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I. STUDIES ON THE BIO-ASSAY OF PITUITARY EXTRACTS:  
CONCERNING THE USE OF A DESICCATED INFUN-  
DIBULAR POWDER AS A STANDARD IN THE  
PHYSIOLOGICAL EVALUATION OF  
PITUITARY EXTRACTS

By MAURICE I. SMITH and WM. T. McCLOSKEY

II. SOME FACTORS CONCERNED IN THE DETERIORATION  
OF PITUITARY EXTRACTS

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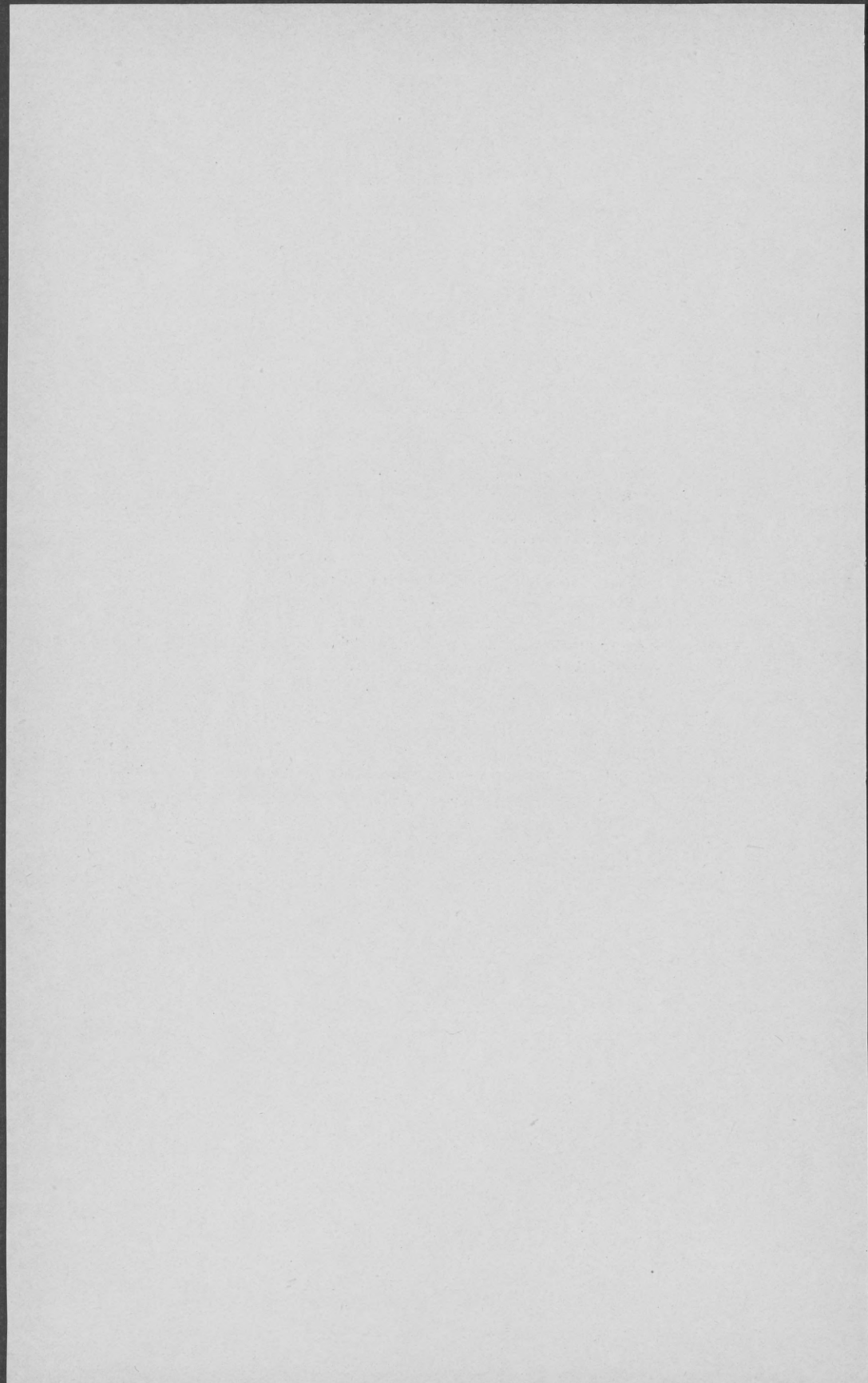
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# **STUDIES ON THE BIO-ASSAY OF PITUITARY EXTRACTS: CONCERNING THE USE OF A DESICCATED INFUNDIBU- LAR POWDER AS A STANDARD IN THE PHYSIOLOGICAL EVALUATION OF PITUITARY EXTRACTS.<sup>1</sup>**

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## **INTRODUCTION.**

It is needless to enter here into a discussion concerning the necessity of accurately standardizing extracts prepared from the infundibular lobe of the pituitary gland, to insure uniformity in potency of the various commercial products. This has been sufficiently emphasized by various workers in this field, and the matter has been considered of so great importance that the ninth revision of the United States Pharmacopœia adopted a method for the bio-assay of pituitary extracts and made it obligatory that the commercial products conform to a certain standard (1). Although several years have elapsed since this went into effect, pituitary extracts from various commercial houses to-day show as much variation in potency as prevailed before the method of assay was adopted. Obviously, either the commercial firms failed to adhere to the prescribed standard, or the standard or method is unreliable. It appears that there is truth in both deductions. Several years of experience with this method of assay of pituitary extracts convinced workers in this field of the unreliability of the standard (Beta-aminazolyethylamine, or briefly, histamine) originally proposed by Roth in 1914 (2). Expressions of dissatisfaction with the standard are found in the publications of Fenger (3), Pittenger and Vanderkleed (4), Eckler (5), and others. In 1918 Spaeth wrote in reference to this standard that his "experiments confirm the evidence that histamine, on account of its deterioration and for other reasons, is not a practical standard" (6). Consequently we find that a commercial firm standardizes its pituitary extracts by a method which it finds most suitable, without regard for what other manufacturers do, and thus one firm puts out extracts of a potency that bears no relation whatever to that of extracts made by other firms.

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<sup>1</sup> Manuscript submitted for publication March 18, 1923.

In 1918 Spaeth (6), not being satisfied with histamine as a standard for the bio-assay of pituitary extracts, suggested potassium chloride as a standard. He based this upon his observation that the reaction of the isolated uterus of the guinea pig to potassium chloride is qualitatively of the same order as that to pituitary extracts. Spaeth's experiments led him to believe that there is also a very close quantitative relationship in the reaction of the isolated uterus of the guinea pig to certain concentrations of potassium chloride and of pituitary extracts. This did not find confirmation in the hands of Nelson (7). During the months of March and April, 1922, one of us (M. I. S.) carried out a series of observations on the use of potassium chloride as a standard, and it was found that out of a series of experiments upon the isolated uterus of the guinea pig with a given pituitary extract it was possible to get a number of experiments yielding fairly concordant results, giving a definite ratio of activity between the extract and potassium chloride. Many experiments, however, showed variations from the established ratio all the way up to 500 per cent. It has not been possible to define clearly the conditions under which consistent results could be obtained with uniformity and it was therefore concluded that there is no constant parallelism in the irritability of different uteri or of the same uterus under different conditions toward potassium chloride and pituitary extracts.

Perhaps the clearest argument against the use of both histamine and potassium chloride as standards in the bio-assay of pituitary extracts was brought out recently by Burn and Dale (8) in a publication which appeared at a time when the work detailed in this paper had progressed so far as to enable us to draw some very definite conclusions.

We believe that any attempt at utilizing an artificial standard for the bio-assay of pituitary extracts would be doomed to failure unless it were known definitely that the mechanism of pharmacologic action of the standard and of pituitary extracts on the uterine muscle, the test object, were identical in every respect. This is admittedly impossible in our present state of knowledge, for even if we had definite knowledge of the action of the proposed standard we should have no exact information as to the mode of action of pituitary extracts on the uterine muscle. Much less do we know what influence slight deviations in the composition of the medium bathing the isolated uterine segment might have upon its reactions to pituitary extracts. In our judgment preparations from the pituitary gland alone, and, since the chemistry of its active principles is virtually unknown, only preparations that represent the entire infundibular lobe, should be used as a standard for the physiological assay of

pituitary extracts by the isolated uterus of the guinea pig, which, as is generally recognized, is the most useful test object.

With this in view we have attempted to discover a preparation of the infundibular portion of the pituitary gland which would show a reasonable degree of uniformity in potency, which would keep a reasonable length of time without deterioration, and which could be prepared without an undue amount of chemical manipulation.

#### THE METHOD AND TECHNIC OF THE BIO-ASSAY OF PITUITARY EXTRACTS.

In carrying out the assay of pituitary extracts we have used the method described by Dale and Laidlaw in 1912 (9), with some modifications which we have found very helpful. A diagram and detailed description of the apparatus in our use follows.

The outer warming chamber (A) is of galvanized iron and measures 15 inches in length, 7 inches in width, and  $6\frac{1}{2}$  inches in height. A constant temperature of  $38^{\circ}$  C. is maintained by means of a micro-burner, connected with a Roux bimetallic gas thermoregulator (B). The inner vessel (C) containing Locke's solution and the uterine horn is made of an ordinary Liebig condenser. The lower end serves the purpose of draining off the bathing fluid after the reaction to a given dose of an extract is completed. Through the side tube (D) fresh Locke's solution is allowed to run in. This solution is warmed to the required temperature in the glass coils (E) and a Fresenius nitrogen bulb (F), which are both connected in series with the reservoir of Locke's fluid.<sup>2</sup> The volume of the coils and flask is approximately twice that of the vessel (C), which holds a little over 100 c. c. One end of the horn of the uterus is attached by means of a fine hook to the lower end of the glass tube (G), which is bent down at (H) and drawn out to a capillary tip. The stream of oxygen bubbles conveyed to the fluid in C is thus diverted from the muscle. The Wolff bottle (M) has a capacity of about 500 c. c. and contains a 2 per cent solution of sodium bicarbonate in distilled water. The oxygen passes through this bottle before entering vessel (C).

During the early part of this work we were frequently confronted with the difficulty of the uterine preparation rapidly increasing in sensitiveness toward pituitary extracts, so that successive reactions to a given dose of the extract soon increased to a maximum. This made it impossible to get more than a very few, and often only approximate, comparisons, and at the end of an experiment there would still be some doubt as to the relative potency of the two extracts examined. We suspected that the oxygen which we were using contained some impurity which tended to augment the tone

<sup>2</sup>The senior author had seen a similar warming glass coil arrangement in use in the pharmacological laboratory of the University of Michigan.

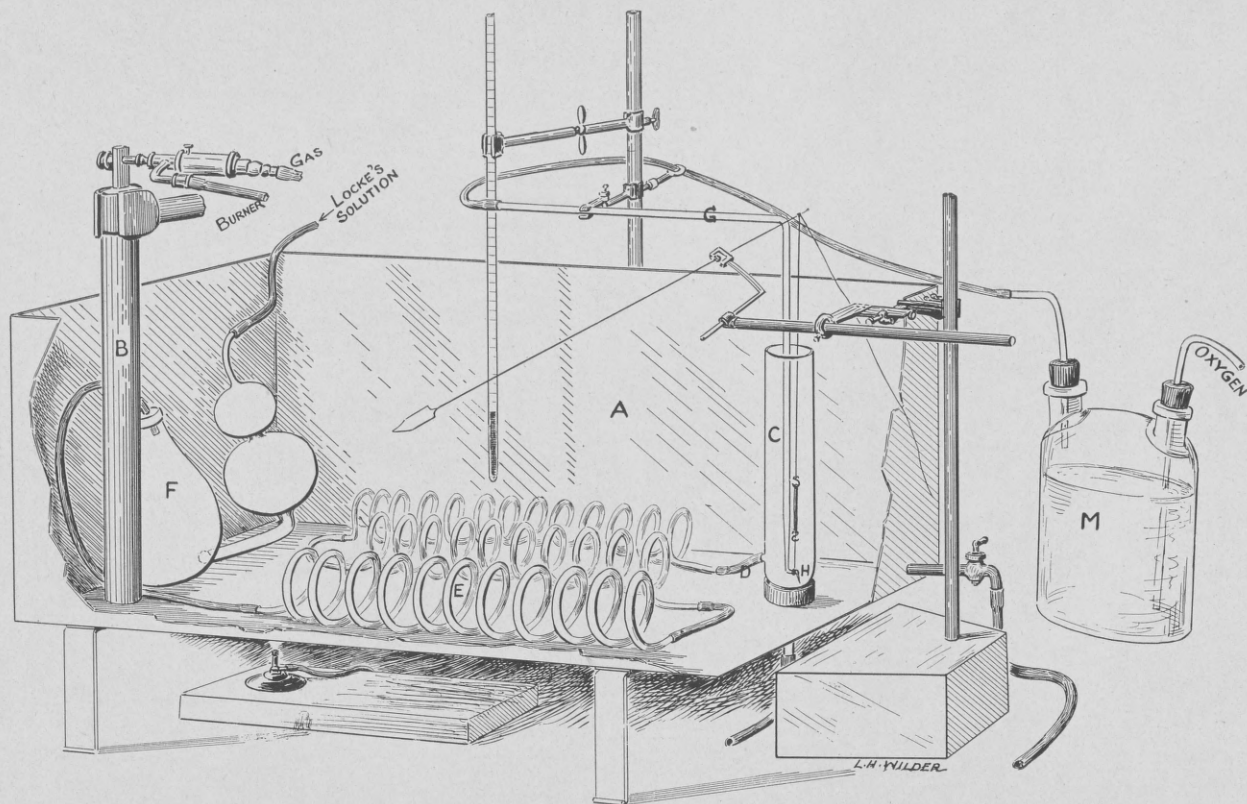


Diagram of perfusion apparatus for the bio-assay of pituitary extracts.

of the uterine muscle and to sensitize it to pituitary extracts. We then attempted to wash the oxygen in an effort to free it of its suspected impurity, and we discovered that by washing the oxygen through a solution of sodium bicarbonate we obtained incomparably better results. We then proceeded to determine by means of indicators, such as are used in hydrogen ion concentration work, whether there was a measurable amount of acidity in the oxygen. The results were negative, but it was observed that as the oxygen bubbled through the solution of sodium bicarbonate a small amount of carbon dioxide was blown over with it, and it was concluded that it is the small amount of carbon dioxide admixed with the oxygen that tends to maintain the tone and irritability of the uterine muscle at a uniform level. Whether the effect is due to the direct action of carbon dioxide on the uterine muscle or to an indirect effect owing to the maintenance of an optimum balance of ions in the bathing fluid can not at present be answered.

We did not attempt to work out in detail the optimum concentration of carbon dioxide. This would obviously require a very large number of experiments. In our experience 500 c. c. of a 2 per cent solution of sodium bicarbonate, renewed every 7 to 10 days, has given us very good results.

We have used throughout this work guinea pigs weighing not much over 250 gm., nor under 180 gm., generally 200 to 240 gm. We are convinced, however, that the age of the animal is a far better guide than its weight. We have frequently met with useless preparations obtained from small guinea pigs, weighing not much over 200 gm., especially if brought into the laboratory from outside breeders. We therefore use as a routine, guinea pigs of our own stock, which are weaned and segregated at the age of 10 to 14 days. At the age of 3 to 5 weeks such guinea pigs yield very useful preparations. It is seldom if ever that uteri obtained from such animals have to be discarded. Trendelenburg and Borgmann (10) have called attention to the great convenience of working with quiescent uteri obtained from young virgin guinea pigs and we can confirm these authors. Such uteri require very little weighting, are easily adjusted, and show a considerable degree of sensitiveness to pituitary extracts.

The animal is killed by a blow on the head, and at once both horns of the uterus are removed and placed in Locke's solution. One entire horn, freed from the broad ligament, ovary, and Fallopian tube is suspended in the bath of Locke's solution at 38° C. We have never seen the necessity of including the ovary and Fallopian tube, as some workers do, but have felt, on the contrary, that it might introduce an error or at least some difficulty in the interpretation of results owing to gradual stretching of the Fallopian tube during the

course of an experiment. Since we know of no special advantage in their use we have uniformly discarded them.

After complete relaxation, which takes from 15 to 30 minutes, the preparation is so adjusted as to write a base line with but slight spontaneous movements. With small uteri of young animals this is readily accomplished by the weight of the lever, or a small additional weight if necessary. We have found that the Harvard aluminum heart lever, with a magnification of approximately 4, serves the purpose very well. This gives a magnification on the tracing of such magnitude as to make it easy to detect small differences in dosage. The extracts to be tested are always prediluted with Locke's solution so that the dose added to the bath of 100 c. c. is about 0.5 c. c., and never exceeds 1.0 c. c. The dilutions are usually made of such strength as to require nearly equal volumes of standard and unknown to elicit equivalent reactions. Each dose is carefully measured with pipettes accurately graduated to 0.01 c. c. After an equivalent dose of the unknown is found for a given dose of the standard, the respective doses are then increased or decreased, or both, by 10 to 20 per cent. This not only confirms the earlier finding, but also gives assurance of the sensitivity of the uterine segment to small increases or decreases of dosage.

The following is the composition of Locke's solution used in this work:

Sodium chloride .....	9.00 gm.
Calcium chloride .....	0.24 gm.
Potassium chloride .....	0.42 gm.
Sodium bicarbonate .....	0.50 gm.
Dextrose .....	0.50 gm.
Glass redistilled water to make .....	1,000.00 c. c.

Our Locke's fluid is freshly prepared each day from stock solutions of twentyfold concentration exclusive of the dextrose. The dextrose is weighed out each time as required.

Burn and Dale (8) recommend a small amount of magnesium chloride to be added to Locke's solution as a means of lessening the tendency to irregular spontaneous rhythm in the uterine muscle. Our method of using oxygen passing through a 2 per cent solution of sodium bicarbonate, as described above, has given us results so gratifying that we have seen no necessity for trying out extensively anything new. We have, however, tried, since the appearance of their paper, a few experiments with Locke's fluid containing the recommended amount of magnesium chloride (0.0005 per cent), and have found it of advantage in some experiments, though in others it seems to have so reduced the sensitivity of the uterine muscle to pituitary extracts that small differences in dosage did not elicit distinct differences in reaction. By our method the uterine muscle

shows very little tendency to irregular spontaneous rhythm, its tone is maintained at a uniform level for hours, and it preserves a high and fairly constant degree of sensitivity toward pituitary extracts.

#### THE STANDARD FOR THE BIO-ASSAY OF PITUITARY EXTRACTS.

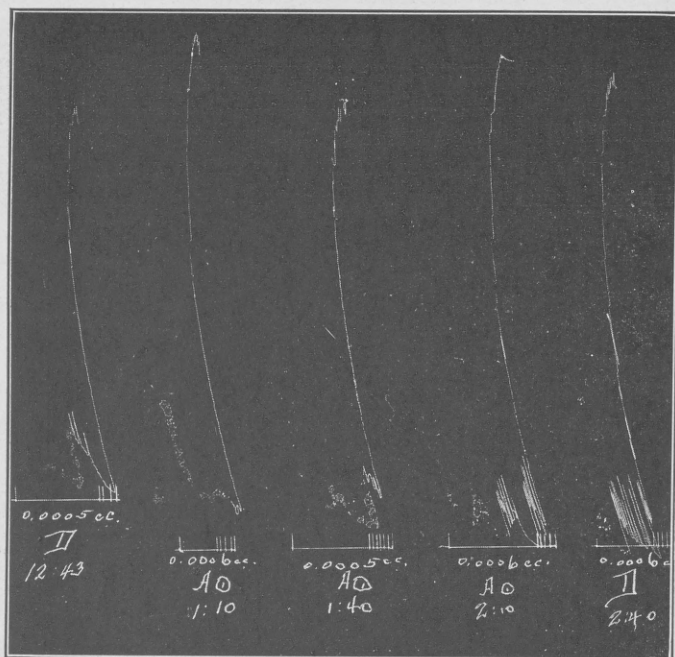
The great variations in activity of commercial extracts, and possibly other considerations must have led earlier workers (2) (6) to the assumption that preparations made from the infundibular lobe of the pituitary gland would lack uniformity and stability, and could not be used as a reliable standard for the assay of pituitary extracts. It is probably such considerations as these that led them to seek artificial standards. The unsatisfactory status of the artificial standards heretofore proposed, as pointed out earlier in this paper, led us to investigate the feasibility of using some preparation of the infundibular portion of the pituitary gland as a standard. Dale and Laidlaw, indeed, made this suggestion in 1912 (9). Obviously, none of the objections raised against artificial standards could be entertained against a preparation from the gland itself, provided, of course, it could be shown that such preparations possess all the activity of the gland, show uniformity in physiological activity, and remain stable over a reasonable length of time.

Since there appears to be some ground for the belief that rapid changes, perhaps autolytic, occur in the posterior lobes of pituitary glands subsequent to their removal we have invariably used fresh material. The glands were in all cases removed within 10 to 20 minutes of the killing of the animal and the posterior lobes were immediately dissected out and worked up according to the methods described below.

It appeared to us that an infundibular powder possessing the requirements as outlined above and yielding its entire activity upon simple extraction should be a most suitable standard for the assay of pituitary extracts. In order to determine the physiological activity of the infundibular powders made by us at various times, a simple acidulated saline extract of fresh infundibular lobes from the same batch was also prepared each time a lot of glands was gathered for the preparation of an infundibular powder. These extracts were made by weighing out accurately a definite amount of fresh posterior lobe material, which was then thoroughly ground to a fine pulp in an agate mortar with a little chemically pure sand, extracted with a measured volume of one-fourth of 1 per cent acetic acid in physiological salt solution, heated quickly to boiling and filtered. The filtrate was at once brought to the laboratory, put into ampoules, sterilized in steam at 100° C. on three successive days for 20 minutes each, and stored in the ice box. These fresh gland ex-

tracts were uniformly made to represent 5 per cent of fresh posterior lobe material.

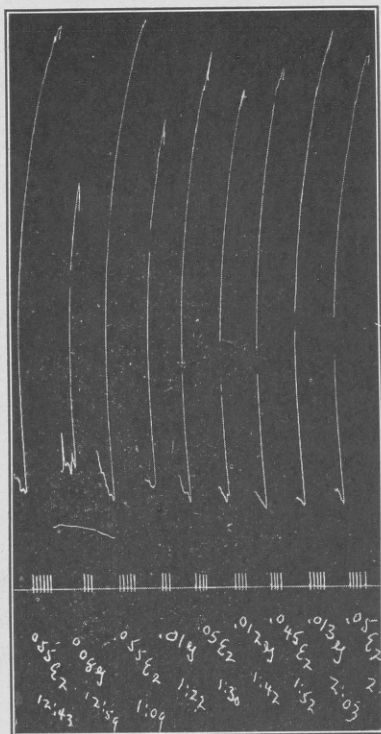
Primarily these extracts were made to serve as a standard of comparison of the activity of the respective infundibular powders. We have had occasion, however, to assay against each other eight such extracts made at different times during the months of June to October, 1922, and, contrary to our expectations, we were much impressed with the remarkable uniformity in their potency. Tracing



TRACING 1.—July 13, 1922. Guinea pig, 210 grams. Shows equality of 5 per cent fresh gland preparations A1 and D. In this, as in all subsequent tracings (except 5), the time is marked in minutes, and indicates the interval between adding the dose to the bath and replacing it with fresh Locke's solution.

1 shows the uniformity in activity of two such extracts, A 1 made in June and D prepared in July. We had, however, one notable exception to this. Five per cent fresh gland extract G, made by one of us in August with the same technic as the others showed only half the theoretical strength when assayed at the time of its preparation against extract D already referred to. Later the same extract was also assayed against one of our desiccated infundibular preparations E 2 and again was found to represent only 26.6 mg. fresh gland substance per c. c. (See tracing 2.) We are at a loss to explain this marked divergence.

Since the appearance of the recent publication of Burn and Dale (8) we have made some experiments to determine whether it would be possible to make 10 per cent extracts of uniform potency from fresh frozen posterior lobes by their technic.<sup>3</sup> A batch of fresh pituitary glands was obtained, which immediately upon removal were placed in a freezing mixture. They were brought in the frozen state to the laboratory, the posterior lobes carefully dissected out and minced

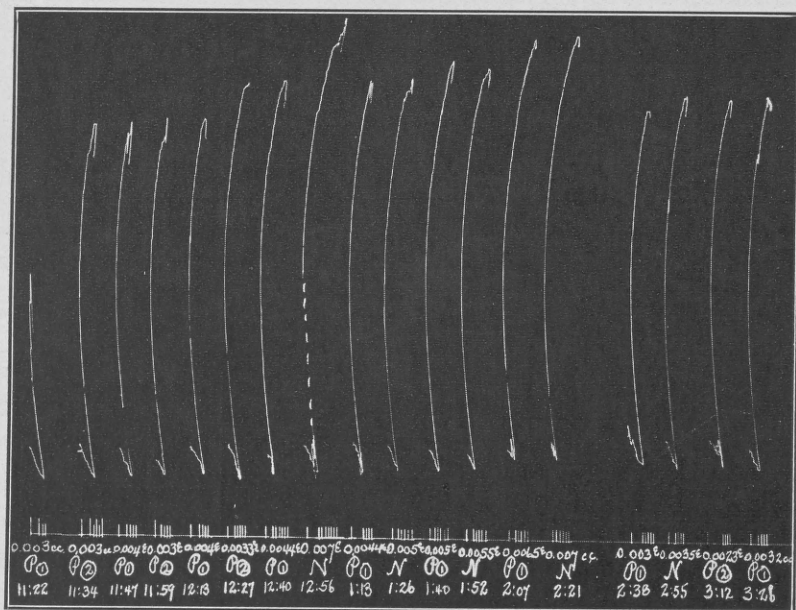


TRACING 2.—December 11, 1922. Uterus of guinea pig, 220 grams. Assay of 5 per cent fresh gland extract G against desiccated infundibular preparation E2. Result: 0.013 c. c. G = 0.05 mg. E2, or 1.0 c. c. G = 3.8 mgs. E2. Hence, 1.0 c. c. G =  $3.8 \times 7 = 26.6$  mgs. fresh gland substance. (1 mg. powder = 7 mgs. fresh gland substance, as shown in Table I.)

finely with scissors. After mixing the whole mass of minced glands, two portions of 4.0 gm. each were weighed out, and two extracts made according to the details of the technic of Burn and Dale. The extracts were placed in ampoules and sterilized on three successive days for 20 minutes each in the Arnold sterilizer. These extracts,

<sup>3</sup> Burn and Dale made their extracts to represent 2.5 per cent of infundibular material. We made 10 per cent extracts in conformity with the recommendations of the subcommittee on bio-assay of the tenth revision of the United States Pharmacopœia. This however has undergone revision since the completion of these experiments, a 5 per cent extract having been substituted by the subcommittee.

designated P 1 and P 2, respectively, were later assayed against each other as well as against a 5 per cent fresh gland extract N, made by our method of extraction from the same material that P 1 and P 2 were made. The results of this assay are shown in tracing 3, from which it appears that the activity of extracts P 1 and P 2 is in the ratio of 3:4, the former representing the activity of 55 mg., and the latter 73.3 mg. of fresh gland substance per c. c. In a confirmatory assay of extract P 1 against one of our desiccated infun-



TRACING 3.—January 22, 1923. Guinea pig, 220 grams. P1 and P2 = 10 per cent extracts of minced fresh frozen posterior lobes of the pituitary, sterilized in the usual manner. N = 5 per cent extract of fresh frozen glands of same batch as above, made in the usual manner. Result: 0.003 c. c. P2 = 0.004 c. c. P1, and 0.005 c. c. P1 = 0.0055 c. c. N. Hence, 1 c. c. P1 = 1.1 c. c. N = 55.0 mgs. fresh gland substance, and 1 c. c. P2 =  $55.0 \times \frac{4}{3} = 73.3$  mgs. fresh gland substance.

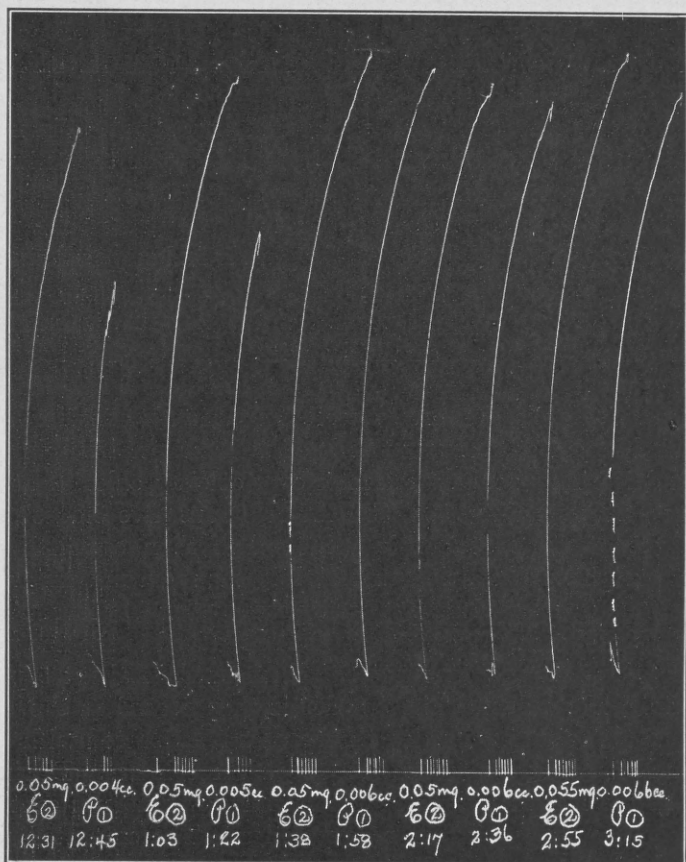
dibular powders E 2, one c. c. was found equal in activity to that of 8.3 mg. of the powder or 58.1 mg. fresh gland substance, since, as it will appear from Table I, approximately 7 mg. of fresh gland substance is the equivalent of 1 mg. of our powders. (See tracing 4.)

In the light of the work of Adams (11), to which reference will be made later, the possibility of sterilization affecting the activity of extracts P 1 and P 2 was considered, since their acidity is relatively low (about 0.07 per cent acetic acid<sup>4</sup>).

Another experiment was therefore carried out in which two extracts were made from the same batch of minced material. To the one acetic acid was added to make approximately 0.07 per cent and to the other

<sup>4</sup>As recommended by the subcommittee on revision. This has also been recently changed to 0.3 per cent glacial acetic acid.

sufficient to make 0.25 per cent. The two extracts were sterilized by fractional sterilization in the Arnold sterilizer and assayed against infundibular powder E 2. The former extract was found to represent the activity of about 40 mg. of fresh gland substance per c. c., and the latter 60 mg. Evidently low acidity, while a probable factor in reducing the potency of the extracts during frac-



TRACING 4.—January 19, 1923. Guinea pig, 240 grams. E2 = extract of desiccated infundibular preparation E2. P1 = 10 per cent extract of minced fresh frozen posterior lobes of the pituitary, sterilized in the usual manner. Result: 0.006 c. c. P1 = 0.05 mg. E2. Hence, 1 c. c. P1 = 8.3 mgs.  $E2 \times 7 = 58.1$  mgs. fresh gland substance.

tional sterilization, does not entirely account for the relatively low activity.

This serves to emphasize the point which Burn and Dale (8) stressed, viz, the great care that has to be practiced in the preparation of extracts from the fresh infundibular lobe of the pituitary gland, and the importance of adhering to strict details in their preparation, if they are to be made of uniform activity, the prime

requisite of a standard. In view of the great difficulty of making such extracts of uniform activity even with the best of technic and where conditions are most carefully controlled, it seems to us that a liquid "standard extract" is not an ideal standard for the bio-assay of commercial extracts.

The infundibular powders which we have found to possess a high degree of uniformity in activity, which yield upon simple extraction the activity of the whole gland, and which have suffered no deterioration up to this time (a period of nearly nine months), were made in the following manner.

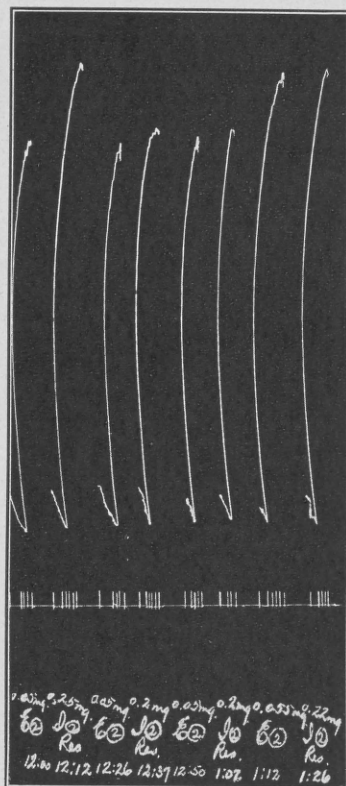
The carefully dissected fresh posterior lobes, about 40 in number, obtained within 10 to 20 minutes of the killing of the animal, are dropped at once into a flask containing about 150 c. c. of acetone.<sup>5</sup> Forty to sixty glands will suffice for 1.5 to 2 gm. of the powder. This treatment rapidly dehydrates the glands and stops all enzymatic action. The glands are then brought to the laboratory. At this time they are shrunken and present a tough leathery consistency. They are then cut up into small bits with scissors, placed in a fresh portion of acetone, and put away in the ice box. By the following morning the glands are pretty well dehydrated, considerably defatted, and have lost over two-thirds of their original weight. They are then dried in a vacuum desiccator over calcium chloride at a temperature not exceeding 50° C. After about five hours' drying the weight of the material is a little less than one-fifth of the original weight. The dried material is then ground in an agate mortar to a fine powder that will pass a No. 40 sieve. A small amount of residue remains that can not be reduced to the fine powder, but generally does not constitute much more than about 5 per cent of the finished product. The powder is then placed in the vacuum desiccator. The following day the powder is extracted with acetone in a small Soxhlet apparatus for about three hours. During this operation the loss in weight in one instance was 15 per cent of the weight of the powder prior to extraction. The extracted powder is then dried in vacuo over night at a temperature of about 40° C. to constant weight. The powder, after it is thus reduced to a constant weight, represents approximately 16 per cent of the original weight of the fresh glands. The finished powders are kept in the laboratory out of the light in a vacuum desiccator charged with calcium chloride.

The residue remaining after the material is reduced to a fine powder represents only part of the activity of the powder. This was determined for desiccated preparations H 2 (Table I) and I 2. In the latter instance 85 mg. of residue, or 6 per cent of the finished

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<sup>5</sup> The fresh glands representing the powders described in Table I were weighed just before they were placed in the acetone.

product, were obtained. An extract of the entire residue was made, and this was assayed against an extract of desiccated infundibular preparation E 2. Inspection of tracing 5 shows that the residue represents only about one-fourth of the activity of the powder. Likewise the entire residue of desiccated infundibular preparation H 2 was extracted and assayed against our "standard extract D,"



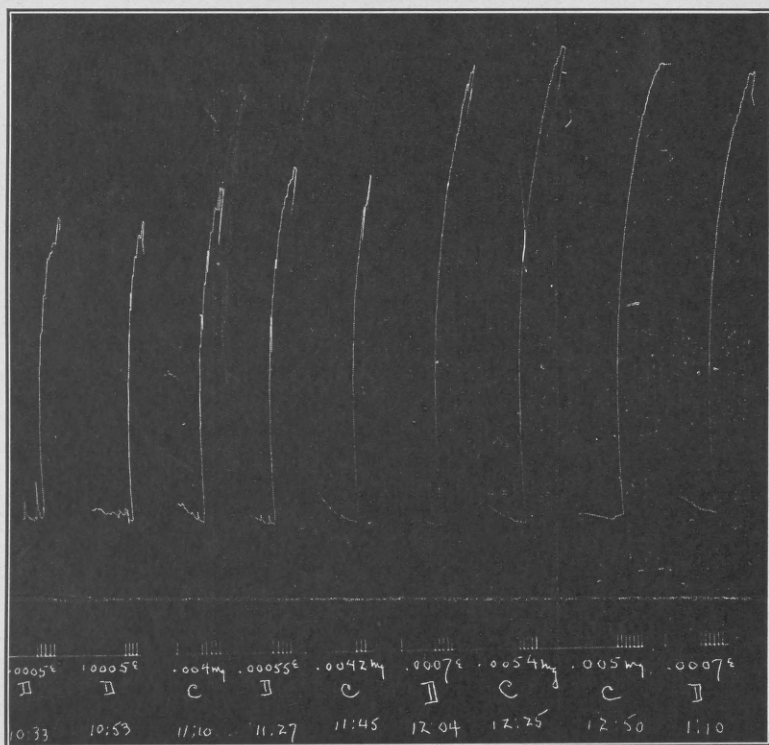
TRACING 5.—January 13, 1923. Guinea pig, 240 grams. Assay of residue obtained from desiccated infundibular preparation I2 against desiccated infundibular preparation E2. 0.2 mg. residue I2 = 0.05 mg. E2; hence, total residue of I2 (85 mgs.) = 21.2 mgs. of active powder.

to which we shall refer later, and it was found to represent about one-fifth of the activity of the corresponding powder.

At the beginning of the work it seemed desirable to have some definite uniform standard for the determination of the activity of the infundibular powders which we were planning to make from time to time. For this purpose 100 c. c. of a 5 per cent fresh gland extract were carefully prepared early in July, placed in ampoules, sterilized in the manner already described, and stored in the ice box. This we designated "standard extract D," and the activity of the six desic-

cated infundibular preparations made during the months of June to October (see table I) was determined and expressed in terms of milligrams of fresh gland substance represented in extract D.

The extracts of the desiccated and defatted infundibular preparations are made as follows. Exactly 10 mg. of the powder are weighed out accurately and transferred to an agate mortar. Exactly 10 c. c. of one-fourth of 1 per cent acetic acid in distilled water are measured



TRACING 6.—October 16, 1922. Uterus of guinea pig weighing 200 grams. Assay of desiccated infundibular preparation C against fresh gland extract D. Result: 0.0042 mg. C = 0.00055 c. c. D, and 0.005 mg. C = 0.0007 c. c. D; thus, 1 mg. C = 0.135 c. c. D. Hence, 1 mg. C =  $0.135 \times 50 = 6.8$  mgs. fresh gland substance.

into a pyrex test tube of convenient size. The powder in the mortar is moistened with a drop or two of the solvent and is then thoroughly triturated to an impalpable frothy consistence. The remainder of the solvent is then added gradually and the mixture is thoroughly stirred for several minutes. This yields an opalescent solution, which is transferred back to the test tube, heated to boiling, and filtered. The clear filtrate thus represents the activity of 1 mg. of powder per cubic centimeter of solvent. Although we had invariably used this technic for extraction of the desiccated infundibu-

lar preparations, we were able to show subsequently that a much simpler method of extraction is equally efficacious. We shall refer to this again later.

The results of the assays as well as some other pertinent facts concerning the six desiccated and defatted infundibular preparations made during the months of June, July, August, and October are given in Table I. It will be remembered that the assays were made against 5 per cent fresh gland extract D. By way of illustration tracing 6 is appended showing one of the four experiments made to estimate the activity of infundibular powder C.

TABLE I.—Potency of desiccated and defatted infundibular preparations of the pituitary gland.

Preparation No.	Time of preparation 1922.	One mg. of powdered preparation=mg. of fresh gland.				Potency of preparation (average by assay by actual weight).	Residue per cent of powdered preparation.	
		By assay.			By actual weight.			
		Experiment No.	Mg.	Average.				Variation per cent.
A 2-----	June-----	1	7.0			<sup>1</sup> 6.0	<sup>1</sup> 120	( <sup>2</sup> )
		2	7.0		+4			
		3	7.5	7.2	-1			
		4	7.1					
C-----	do-----	1	6.8			( <sup>2</sup> )		
		2	7.5		+4			
		3	6.9	7.2	-5			
		4	7.5					
E 2-----	July-----	1	5.7			6.3	107	5
		2	6.7		+23			
		3	6.3	6.7	-14			
		4	6.6					
		5	8.3					
F 2-----	do-----	1	6.7			6.2	108	7
		2	6.7		+8			
		3	6.3	6.7				
		4	6.7		-6			
		5	7.2					
G 2-----	August-----	1	8.3			7.4	101	16
		2	7.8					
		3	6.9	7.5	+10			
		4	7.5		-8			
		5	6.9					
H 2-----	October-----	1	7.4			6.3	118	8
		2	7.5	7.4	+2			
		3	7.1		-3			
		4	7.4					

<sup>1</sup> Approximately.

<sup>2</sup> Not determined.

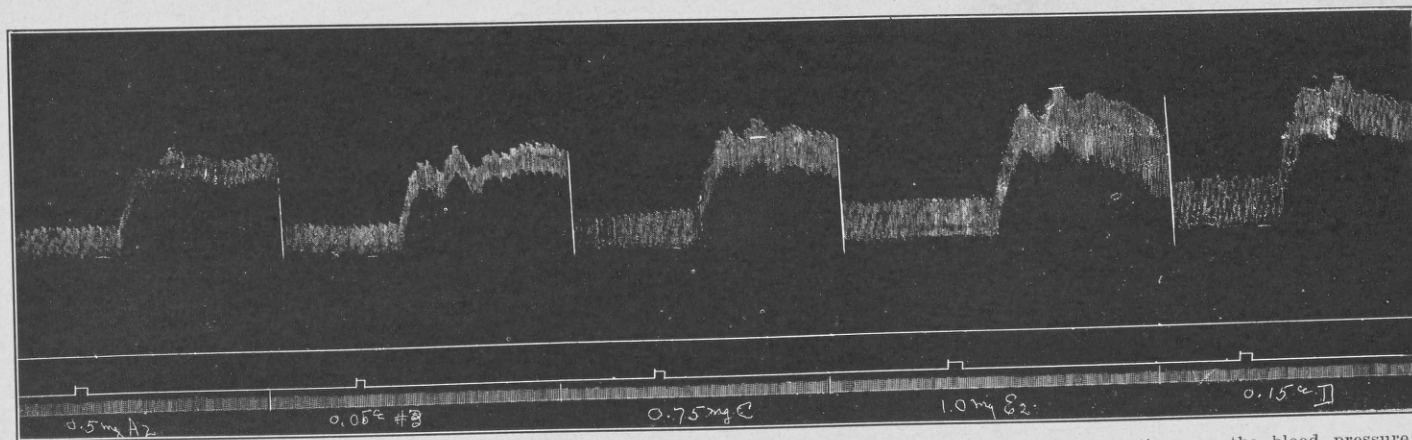
It will appear upon examination of Table I that though the variation of an individual assay of a given powder may be over 20 per cent, the average value of activity of 1 mg. of the several powders is approximately equivalent to that of 7.0 mg. of fresh gland substance, with a variation of less than 5 per cent from the average. It is further of interest to note that the activity of the powders by assay is somewhat higher than their value by actual weight. Thus 1 mg. of the desiccated and defatted powders represents a little over 6 mg. of fresh gland substance by actual weight, while by assay against

D, approximately 7 mg. The relatively high figure for powder G 2 (7.4 mg.) is due to the large amount of discarded residue which undoubtedly could have been reduced to the usual percentage by further grinding. The slight and constant disproportion of the value of the powders as obtained by assay and by actual weight gives them an activity of a little over 100 per cent of that of the whole gland. This we believe is due to the more thorough and complete extraction of the powder than is possible in the case of the fresh gland.

It is therefore evident from the figures given that fresh infundibular material, dehydrated, desiccated, and defatted as described yields powdered preparations of remarkably uniform potency, representing the entire activity of the whole gland, at least in so far as concerns the oxytocic principle.

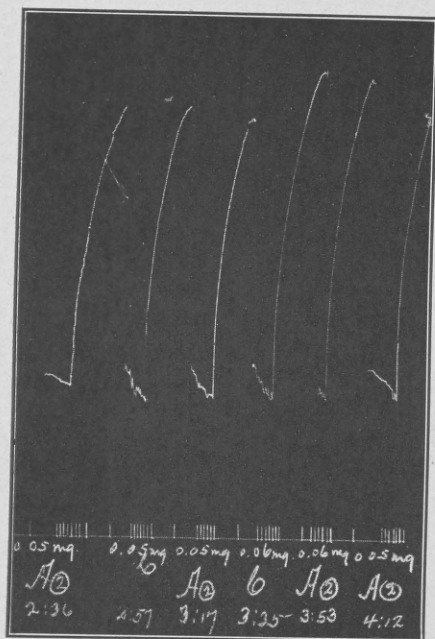
In order to further assure ourselves that our desiccated preparations represent the whole activity of the gland, several experiments were made to determine the activity of these preparations as regards the pressor principle. These experiments were carried out upon dogs under morphine and chloretone anaesthesia. Atropine was injected intravenously, to eliminate vagal effects. The extracts were injected into the femoral vein and the effect on the blood pressure recorded. By giving small doses of the extracts sufficiently far apart, i. e., at 15 to 25 minute intervals, it is possible in our limited experience to get a sufficiently large series of consistent reactions to give one a very fair idea of the relative pressor activity of two or more preparations. The blood pressure method, we admit, does not detect differences in the pressor activity with as fine a sensitiveness as the isolated uterus detects differences in the oxytocic activity. Our experiments, nevertheless, were sufficiently definite and the results clearly indicate a pressor activity for our desiccated infundibular preparations quite consistent with their oxytocic activity as determined by the isolated uterus method. Reference to tracing 7 will show that 1.0 mg. of powder E 2 is equal in pressor activity to 0.15 c. c. 5 per cent extract D or 7.5 mg. fresh gland substance. Similarly the reaction to 0.5 mg. powder A 2 is exactly equal to that of 0.05 c. c. commercial extract No. 3, thus making 1 mg. of the powder equivalent in its pressor action to 0.1 c. c. of commercial extract No. 3. This ratio is quite in keeping with that for their oxytocic activity as will be shown later (see Table II).

As a further check upon the uniformity in activity of the infundibular powders, numerous assays were made at different times against each other. The results of some of these experiments are illustrated by tracings 8 to 14. Tracing 8 shows that defatted infundibular powder A 2 is equal in oxytocic activity to that of C. These are our earliest preparations which were made in June, 1922. Trac-

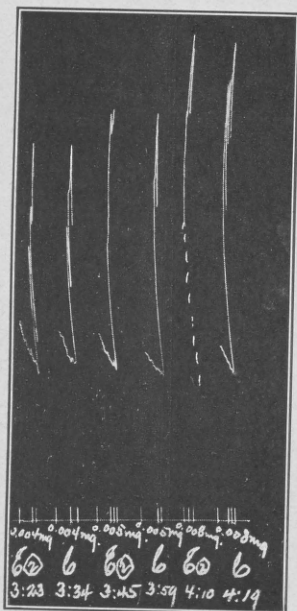


TRACING 7.—Dog, female, 6.2 kgs. morphine and chloretone. Effect of extracts of desiccated infundibular preparations on the blood pressure. The pressor action of 0.5 mg. A2 = that of 0.05 c. c. commercial preparation No. 3, thus making 1 c. c. of the latter equivalent to 10 mgs. of the infundibular preparation (cf. Chart 1). The reaction to 1.0 mg. of infundibular preparation E2 is equal to that of 0.15 c. c. D, hence the pressor action of 1.0 c. c. of fresh gland preparation D is equal to that of 6.7 mgs. of desiccated infundibular preparation E2 (cf. Chart 1).

ing 9 likewise shows no detectable difference in activity between infundibular powders C and E 2, the former having been made in June and the latter in July. Tracing 10 shows the result of an assay carried out in November upon infundibular powders F 2 and H 2. The former was made in July and the latter in October. The difference in activity, if any, is slight and insignificant, as it appears to be no greater than 5 per cent. Tracing 11 shows the result of an assay carried out in December upon infundibular powders E 2 and



TRACING 8.—Nov. 10, 1922. Guinea pig 170 grams. Shows equality of infundibular desiccated preparations A2 and C. Both prepared in June.

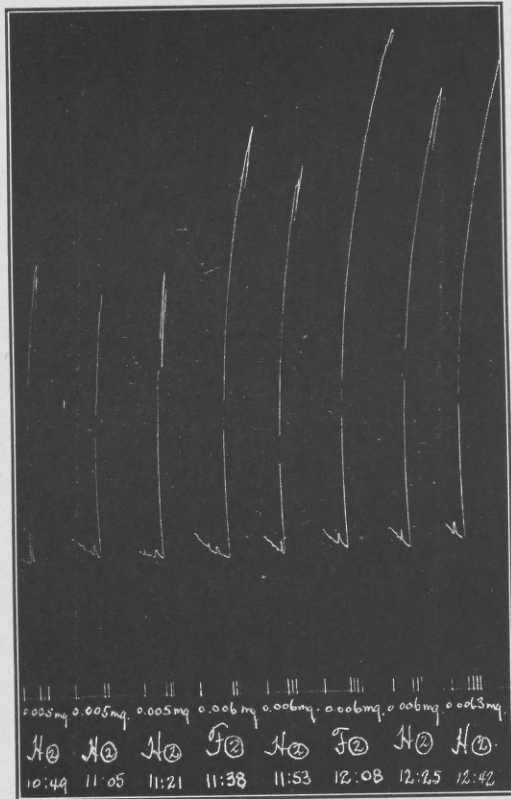


TRACING 9.—November 18, 1922. Guinea pig, 225 grams. Shows equality of infundibular desiccated preparations E2 and C. C was prepared in June, E2 in July.

G 2 made in July and August, respectively. These preparations are likewise shown to be equal in potency. The above tracings thus show equality in oxytocic activity of the desiccated infundibular preparations described in Table I, where they are also shown to be equal in activity when assayed against fresh gland extract D.

Since the completion of the work summarized in Table I another desiccated and defatted infundibular powder was made in the same manner as the others from glands gathered in December. This was designated I 2. This infundibular powder was assayed against

fresh gland extract D and the results indicated the same value for this powder as had been obtained for the others. This incidentally also showed that fresh gland extract D had not undergone any deterioration in five months. Tracings 12 and 13 show that infundibular powder I 2 made in December shows no appreciable difference in oxytocic activity from powders A 2 made in June and F 2 made in July.



TRACING 10.—November 22, 1922. Guinea pig, 190 grams. Shows approximate equality of infundibular desiccated preparations F2, made in July, and H2, made in October. The difference in potency of the two preparations does not exceed 5 per cent.

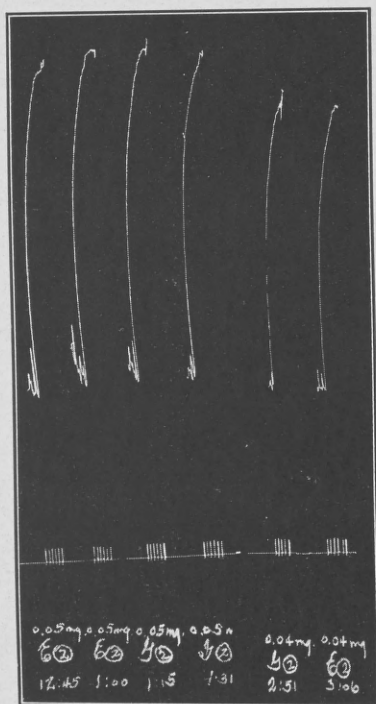
Another infundibular powder J 2 made recently in the same manner as the others from glands gathered in February and assayed against several of the earlier ones was likewise found to be of the same activity. Tracing 14 shows that powder J 2 made in February, 1923, is practically equal in activity to A 2 made in June, 1922.

The eight infundibular powders made during the months of June, 1922, to February, 1923, are thus shown to be uniform in activity, to represent the entire oxytocic and pressor activity of

the gland, to be free from seasonal variations in activity, in so far as our observations have gone, and to suffer no deterioration within a period of nearly nine months.

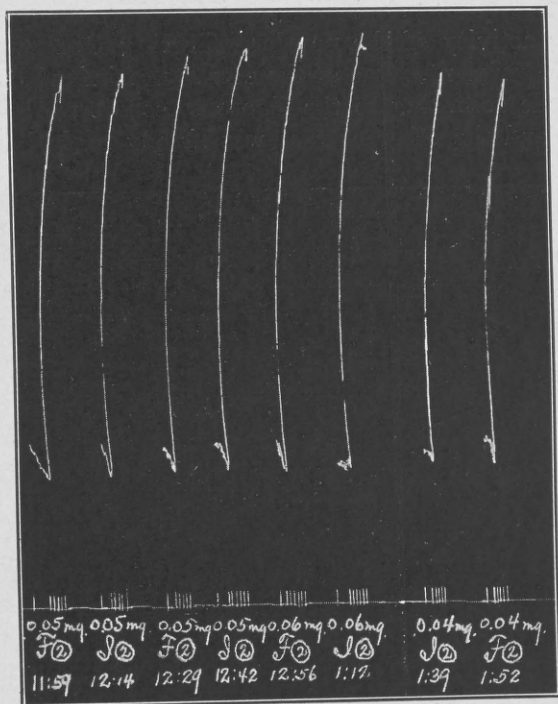
#### THE EXTRACTION OF THE DESICCATED AND DEFATTED INFUNDIBULAR PREPARATIONS.

During the major part of this work it was assumed that a very careful technic in the extraction should constitute an important factor in extracting all the activity of the infundibular powders.

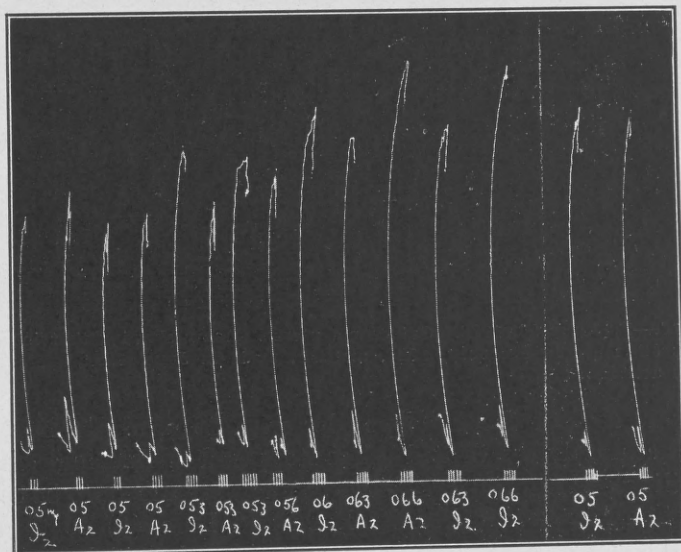


TRACING 11.—December 12, 1922. Guinea pig uterus. Shows equality of infundibular desiccated preparations E2 and G2. The former was prepared in July and the latter in August.

We have therefore uniformly employed the method of extraction previously described, the solvent being one-fourth of 1 per cent acetic acid in distilled water. Subsequently experiments were made to determine what factors if any are particularly concerned in the extraction of the oxytocic principle from our specially prepared powders. As a result of a series of tests it was found that simply vigorous shaking of the powder with distilled water in the proportion of 10 mg. of the powder to 10 c. c. of water for about 10 minutes,

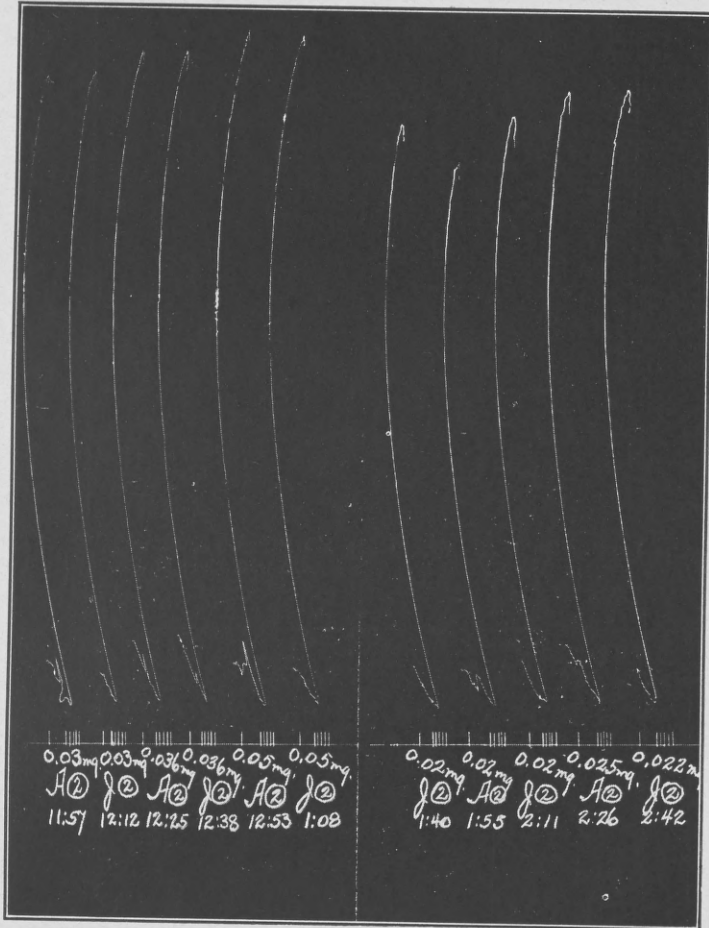


TRACING 12.—January 23, 1923. Guinea pig, 190 grams. Shows equality of desiccated infundibular preparations F2 and I2. The former was made in July and the latter in December, 1922.



TRACING 13.—December 16, 1922. Guinea pig, 200 grams. Shows equality of infundibular desiccated preparations A2 and I2. A2 was made from glands gathered in June; I2 was made from glands gathered in December.

then heating it quickly to boiling and filtering, will extract all of the oxytocic activity of the powder. Tracing 15 shows the result of such an experiment. One of the extracts of infundibular powder E 2 was made according to the rigorous technic we had generally been using, and the other extract was simply made by vigorously

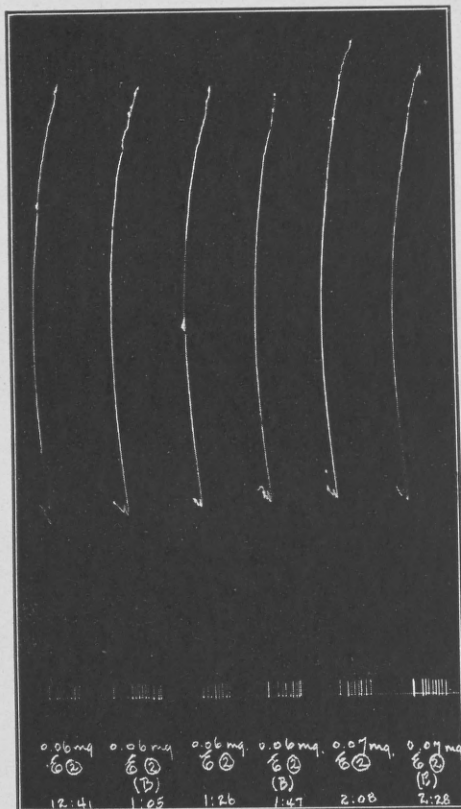


TRACING 14.—February 23, 1923. Guinea pig uterus. Assay showing that infundibular powders J2, made in February, 1923, and A2, made in June, 1922, are practically equal in activity.

shaking 10 mg. of the same powder with 10 c. c. distilled water in an accurately graduated glass-stoppered measuring cylinder, then boiling and filtering. The two extracts are shown to be equal.

Attention has been called by Burn and Dale (8) to the rapid deterioration in activity of fresh posterior lobes if kept for any length of time even at room temperature, and great stress is laid by them upon the minute details which must be practiced in the prepa-

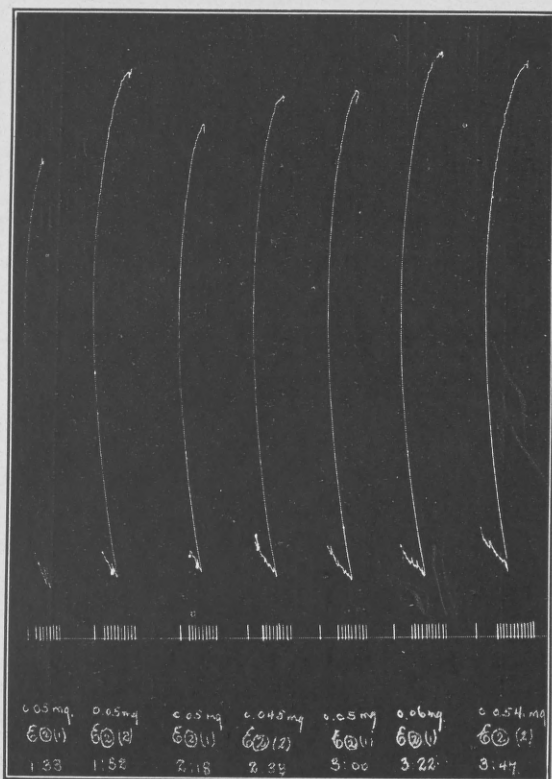
ration of extracts from fresh posterior lobes, in order to eliminate deterioration in activity from this cause. We have made some experiments to determine the effects of brief exposures at temperatures up to 38° C. on the activity of unboiled extracts of our infundibular powders. Extracts of the infundibular powders were prepared by our usual technic, but without boiling were left at room temperature



TRACING 15.—January 9, 1923. Guinea pig, 230 grams. Shows that vigorous shaking of desiccated infundibular preparations with distilled water for a few minutes will effect complete extraction of the oxytocic principle. E2, made in the usual manner (see text); E2 (B), made by shaking vigorously 10 mgs. of E2 powder with 10 c. c. distilled water for about 10 minutes, then boiled and filtered. Note the equality of the two extracts.

for 2 hours, or in the incubator at 38° C. for 2 and 28 hours, respectively. The extracts were then boiled, filtered and assayed against similar extracts made in the usual manner. No appreciable difference in activity was observed. Tracing 16 shows a difference of only 10 per cent between two extracts of powder E 2, the one made in the usual manner and the other incubated at 38° C. for 28 hours before boiling. The difference is in favor of the latter, but we are not inclined to attach much significance to a 10 per cent difference. No

difference in activity could be detected between freshly prepared extracts and those exposed to either room temperature or to incubator temperature for two hours before boiling. In our infundibular powders we thus have a preparation which requires no especially difficult technic for the extraction of its oxytocic principle. The matter of acidity of the solvent may of course be a very important factor if the extract is to be subjected to prolonged boiling, as in the



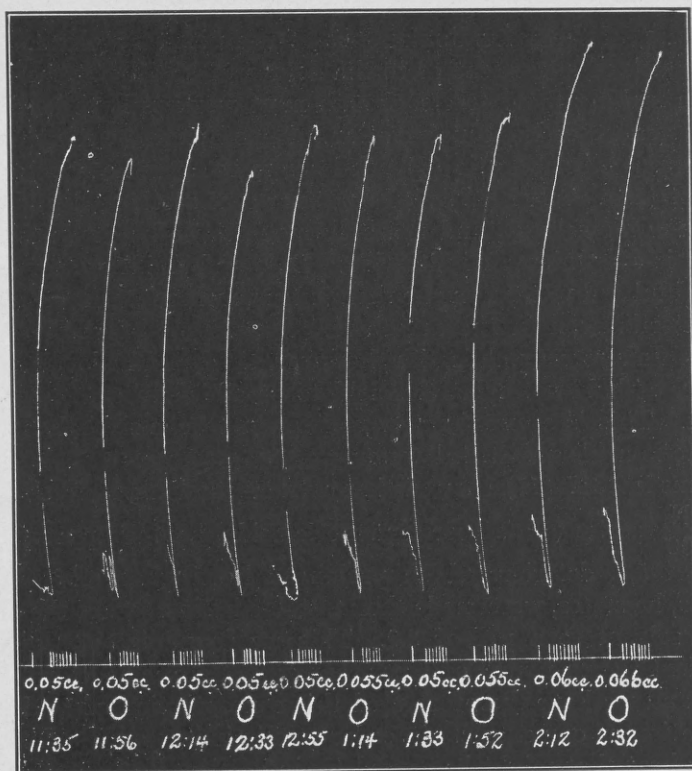
TRACING 16.—December 5, 1922. Guinea pig, 250 grams. Shows that incubation is not a factor in the extraction of the active principle from desiccated infundibular preparations. E2 (1), made in the usual manner (see text); E2 (2), extract incubated at 38° C. for 28 hours, then boiled and filtered. Note the approximate equality of the two extracts, the difference not exceeding 10 per cent.

process of sterilization. This was pointed out by Adams (11), and we shall refer to it later.

#### THE RELATIVE OXYTOMIC ACTIVITY OF POSTERIOR LOBES OF THE PITUITARY FROM STEERS AND FROM COWS.

The material obtained for our work at the local slaughterhouse has been of mixed stock, both steers and cows being represented. We understand that commercial extracts are generally made from glands

of steers. Because of this consideration in particular, apart from the general physiologic interest thereof, an experiment was made to determine whether there is any difference in the oxytocic activity of pituitary glands from males and females. The whole pituitary glands from steers and from cows were placed immediately following their removal in a freezing mixture, and in this condition were



TRACING 17.—January 18, 1923. Guinea pig, 210 grams. Comparison of activity of infundibular extracts made from posterior lobes of pituitary glands of steers and of cows. N, 5 per cent fresh gland extract of posterior lobes from steers; O, similar extract of posterior lobes from cows. Note the approximate equality in potency of the two preparations, the difference not exceeding 10 per cent.

brought to the laboratory. The posterior lobes of the respective batches were then carefully dissected out and immediately worked up into 5 per cent extracts and sterilized by the methods described earlier in the paper. We shall presently show that the oxytocic activity of fresh pituitary glands kept in a frozen condition for several hours is not altered from that of perfectly fresh glands. An assay carried out upon the two 5 per cent extracts against each other showed a difference of not more than 10 per cent. (Tracing 17.) We

conclude therefore that there is no appreciable difference in the oxytocic activity of pituitary glands of steers and cows.

#### THE EFFECT OF STERILIZATION ON THE OXYTOMIC ACTIVITY OF PITUITARY EXTRACTS.

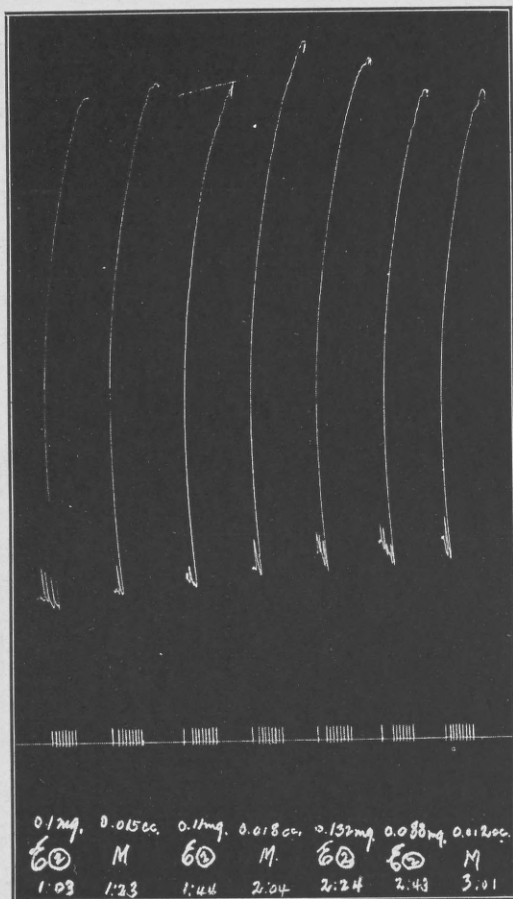
There appears to be no definite agreement as to the method of sterilizing pituitary extracts, nor is there definite information concerning the effects of sterilization upon the active principle, or principles, of the infundibular lobe of the pituitary gland. There is also lack of agreement as to the degree of acidity of the solvent used in making the extracts, though most workers have used acetic acid in greater or less concentration. Thus Fenger at one time used 0.5 per cent glacial acetic (3), while more recently he has used 0.25 per cent (12). Roth (13) used 0.005 per cent acetic acid in distilled water for his solvent, and the only significance he attaches to the acidity is convenience of filtration. Dale and Laidlaw in 1912 (9) used acidulated water to make their extracts which lost some activity upon sterilization in the autoclave. The degree of acidity is not stated. In a recent publication, however, Burn and Dale (8) specify 0.2 c. c. of half normal acetic acid to be added to each 40 c. c of solvent. This is approximately equivalent to one-sixtieth of 1 per cent acetic acid in distilled water.

Adams in 1917 (11) showed that extracts made from fresh glands with  $\frac{N}{60}$  acetic (= 0.1 per cent acetic acid), having approximately a pH of 5.0, deteriorated on prolonged heating at 100° C. The extracts, he states, were made thermostable by increasing the acidity of the solvent to a pH of 3.0. This we found is approximately equivalent to one-fourth of 1 per cent glacial acetic acid in distilled water, and we have therefore used this concentration of acid in the preparation of our extracts in order to eliminate possible deterioration by heat that might be caused by insufficient acidity.

We examined the effects of two methods of sterilization upon the activity of pituitary extracts made with one-quarter of 1 per cent glacial acetic acid in distilled water or physiological salt solution as a solvent; (1) fractional sterilization on three successive days for 20 minutes each in the Arnold sterilizer, and (2) sterilization in the autoclave at 15 pounds pressure. The material was always put into ampoules and sealed. It should be noted that we had been using the former method of sterilization in our routine work, and from our observations had no reason to suspect any change in activity of the extracts from it. It seemed desirable, however, to make some carefully controlled experiments to determine this point. The experiments have shown that fractional sterilization had no effect upon the oxytocic activity of our extracts, while sterilization in the autoclave at 15 pounds pressure caused rapid deterioration of this prin-

ciple, the rate of deterioration being in proportion to the length of time of sterilization.

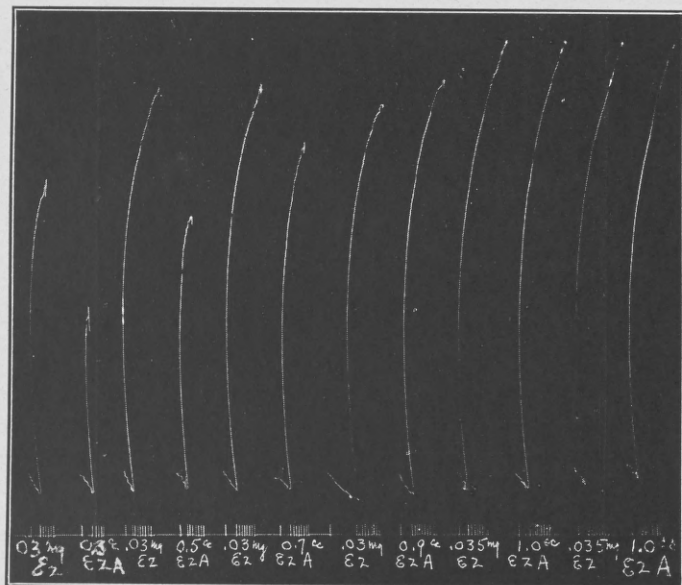
Tracing 18 is a tracing showing no effect on the oxytocic activity of an extract of infundibular powder E 2 made to represent 7 mg.



TRACING 18.—December 6, 1922. Guinea pig, 210 grams. Shows that the oxytocic principle can be extracted completely from desiccated infundibular preparations in concentration equivalent to 5 per cent fresh gland substance, and that steam sterilization on three successive days for 20 minutes does not affect the activity of such extracts. E2, extract of powder made in usual manner (see text); M, extract of E2, 7 mgs. per c. c. of one-fourth of 1 per cent acetic acid in distilled water, ampouled and sterilized in Arnold sterilizer on three successive days for 20 minutes. Result: 0.015 c. c. M = 0.11 mg. E2; also, 0.012 c. c. M = 0.088 mg. E2. Hence, 1 c. c. M = 7.3 mgs. E2.

of powder per c. c. of solvent (equivalent to a 5 per cent fresh gland extract) and sterilized by fractional sterilization, as assayed against an extract of the same powder made in the usual manner without sterilization. The results show no change in oxytocic activity.

Tracing 19 is presented to show the effect of sterilization in the autoclave on the oxytocic activity of pituitary extracts. An extract of infundibular powder E 2 was made in the usual manner. After filtration a portion was reserved for use as standard. The remainder was placed in ampoules, sealed, and sterilized in the autoclave at 15 pounds pressure for a period of 100 minutes. The assay showed that 65 per cent of the oxytocic activity of the sterilized extract was destroyed. A similar experiment showed that 15 minutes sterilization at 15 pounds pressure resulted in a loss of 25 per cent of oxytocic activity.



TRACING 19.—January 10, 1923. Guinea pig, 230 grams. Effect of autoclaving on the activity of infundibular extracts. E2, extract made from powder E2 in the usual manner; E2 (A), portion of same extract autoclaved at 15 pounds pressure for 100 minutes, and diluted 10 times. Result: 0.1 c. c. E2 (A) = 0.035 mg. E2, or 1.0 c. c. E2 (A) = 0.35 mg. E2. Hence, 65 per cent of the active oxytocic principle of E2 (A) extract was destroyed.

It may be concluded therefore that fractional sterilization at 100° C. does not affect the oxytocic principle of pituitary extracts made with one-fourth of 1 per cent glacial acetic acid as a solvent. Sterilization at 15 pounds pressure, even for a brief period, causes some deterioration in activity, the rate of deterioration rapidly increasing with increasing length of time in the autoclave.

#### THE ASSAY OF COMMERCIAL PITUITARY EXTRACTS, USING THE DESICATED INFUNDIBULAR PREPARATIONS AS STANDARD.

Having found our infundibular preparations uniform in potency when assayed against each other as well as when assayed against

fresh gland standard extract D we next examined commercial extracts, using the infundibular powders as standard. Nine commercial extracts purchased in the open market, eight of which were of American make and one of British manufacture, were examined during the months of October and November, 1922. All the ampoules of each lot bore the same serial number, and we were later assured by the respective manufacturers that all ampoules bearing the same serial number had been filled with material from a single extract. Each of the extracts was assayed against each of our six infundibular powders described in Table I. The results of this investigation are summarized in Table II.

TABLE II.—*Assay of commercial pituitary extracts against the desiccated and defatted infundibular preparations.*

Column 1: Fraction of c. c. of commercial extract=1 mg. of desiccated infundibular preparation. Column 2: One c. c. of commercial extract=1 mg. fresh gland substance.]

Infundibular preparations.	Commercial pituitary extracts.																	
	1		2		3		4		5		6		7		8		9	
	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.	c. c.	mg.
A 2-----	{0.30 0.22}	27.7	0.65	11.7	0.11	65.5	0.23	31.3	0.90	8.0	0.34	21.1	0.39	18.5	0.33	21.8	0.16	45.0
C-----	{0.33 0.25}	24.8	{0.50 0.75}	11.6	0.10	72.0	0.23	31.3	0.89	8.1	0.45	16.0	0.35	20.6	0.37	19.5	0.18	40.0
E 2-----	{0.20 0.20}	33.5	{0.80 0.50}	10.5	0.10	67.0	0.23	29.1	0.70	9.6	0.43	15.6	0.55	12.2	0.32	20.9	0.15	44.6
F 2-----	{0.17 0.20}	36.2	{0.60 0.60}	11.3	0.10	67.0	0.20	33.5	0.70	9.6	0.40	16.7	0.48	13.9	0.38	17.7	0.19	35.2
G 2-----	{0.21 0.25}	32.6	{0.60 0.60}	12.3	0.10	75.0	0.20	37.5	0.68	11.0	0.40	18.7	0.43	17.7	0.35	21.4	0.15	50.0
H 2-----	0.20	37.0	0.68	10.9	0.11	67.3	0.23	32.0	0.90	8.2	0.36	20.5	0.45	16.4	0.29	25.5	0.16	46.2
Average mg-----		31.9		11.4		68.9		32.4		9.1		18.1		16.6		21.1		43.5
Variation per cent--		+16		+8		+8		+16		+21		+16		+24		+21		+15
		-22		-8		-5		-10		-12		-14		-26		-16		-19

The commercial extracts examined are numbered in the table from 1 to 9, as it seemed best to omit the names of the manufacturers from the present discussion. In the first column pertaining to each number, the fraction of a cubic centimeter of the commercial extract equivalent in potency to 1 mg. of the respective infundibular powders is given. In the second column pertaining to each number the value of 1 c. c. of the extract is expressed in terms of milligrams of fresh gland substance. These figures are arrived at by simple calculation, bearing in mind that 1 mg. of the infundibular powder is very nearly equal in oxytocic activity to 7 mg. of fresh infundibular substance. At the bottom of each column the average of the six or more assays in milligrams of fresh gland substance per cubic centimeter of extract is given, and the percentage variations of the individual assays from the average are indicated.

It will be seen that there is a wide variation in activity of the commercial extracts, ranging approximately from that of 9 mg. of fresh infundibular substance per cubic centimeter to nearly 70 mg.

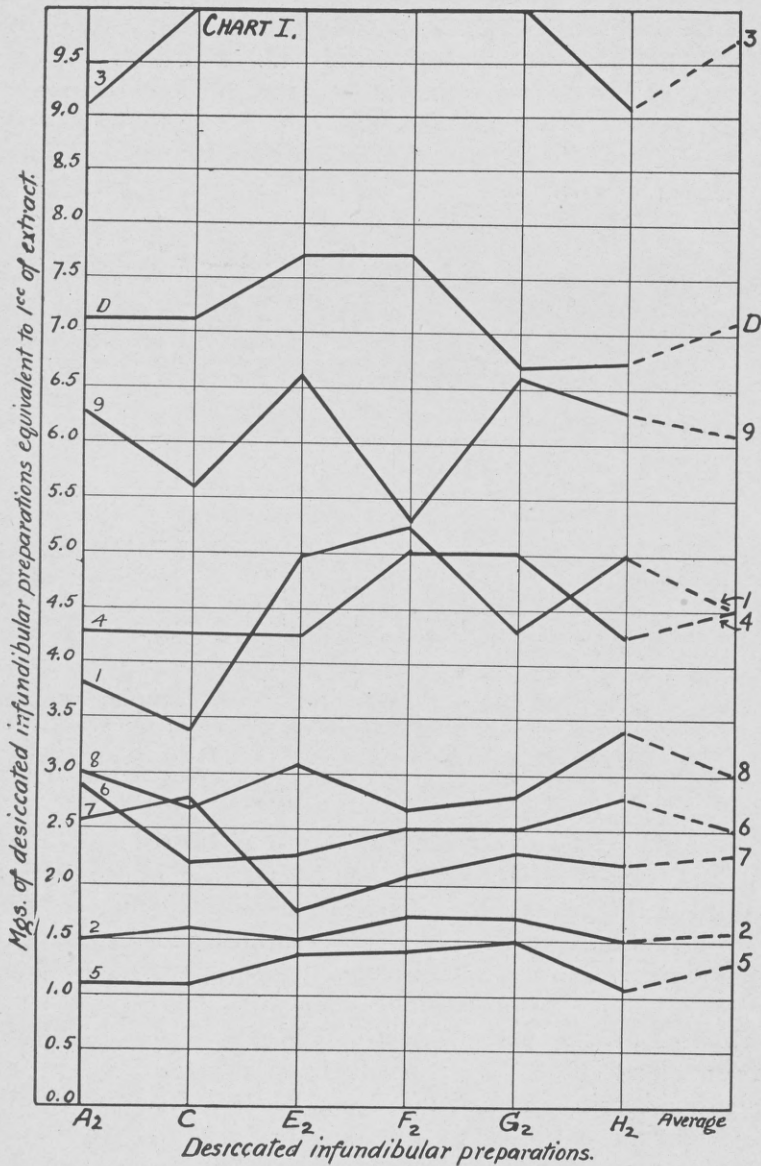


CHART I.—Potency of commercial pituitary extracts expressed in terms of milligrams of desiccated infundibular preparations equivalent to 1 cubic centimeter of extract.

It also appears that several of the extracts assayed at from 2 to 3 per cent of fresh infundibular substance, two at about 1 per cent, two at 4, and one at nearly 7 per cent.

The results summarized in Table II are plotted in Charts I and II. The curves in these charts are formed by joining the points on the scale giving the value of each assay in terms of milligrams of infundibular powder per cubic centimeter of extract as shown

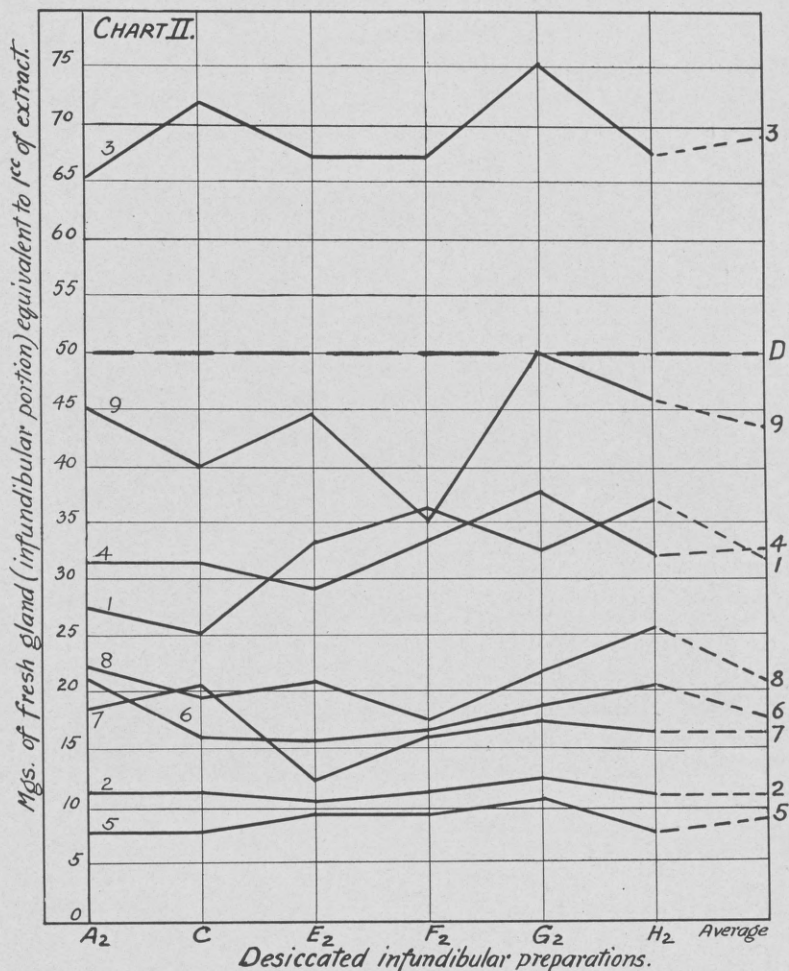
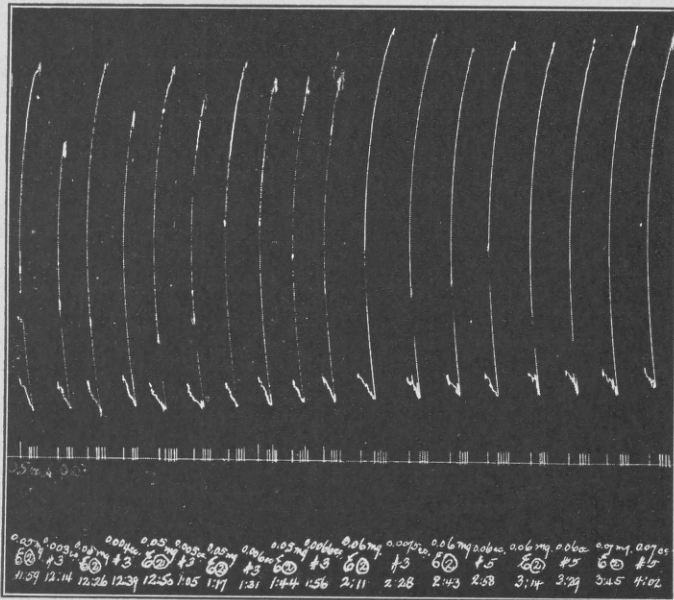


CHART II.—Activity of commercial pituitary extracts assayed against desiccated infundibular preparations, and potency expressed in terms of milligrams of fresh gland equivalent to 1 cubic centimeter of extract.

in Chart I, and in milligrams of fresh infundibular substance per cubic centimeter of extract in Chart II. The last point on the scale in each curve represents the average of the six or more assays. The curves are numbered to correspond to the numbers of the commercial extracts in Table II. Curve D in Chart I represents the value (individual assays and average) of our 5 per cent fresh gland

extract D in terms of activity of milligrams of infundibular powder per cubic centimeter.

As a further illustration of the wide variation in the activity of commercial extracts, tracing 20 shows assays of the weakest (No. 5) and the strongest (No. 3) extracts against infundibular powder E 2. It will appear from the tracing that 1 c. c. of commercial extract No. 3 is equivalent in activity to 8 mg. of the standard, while 1 c. c. of extract No. 5 represents the activity of only 1.0 mg. of the standard. Both commercial extracts assayed January, 1923, at some



TRACING 20.—January 12, 1923. Guinea pig, 240 grams. Assay of commercial extracts Nos. 3 and 5 against desiccated infundibular preparation E2. Result: 0.006 c. c. No. 3 = 0.05 mg. E2, and 0.0075 c. c. No. 3 = 0.06 mg. E2. Hence, 1 c. c. No. 3 = 8.0 mgs. E2, or  $8 \times 7 = 56$  mgs. fresh gland substance. Also, 0.06 c. c. No. 5 = 0.06 mg. E2, and 0.07 c. c. No. 5 = 0.07 mg. E2. Hence, 1 c. c. No. 5 = 1.0 mg. E2, or 7.0 mgs. fresh gland substance; 1 c. c. of No. 3 is therefore equal in activity to 8.0 c. c. of No. 5.

20 per cent below the average found about three months previously, suggesting slight deterioration. The point of greatest interest, however, is the fact that the ratio of activity of the two extracts at this time of assay is 8, essentially the same as it was at the last assay three months previously; in other words, 1 c. c. of commercial extract No. 3 is equivalent in activity to 8 c. c. of commercial extract No. 5.

All commercial extracts examined were labeled "physiologically standardized," a term now obviously without meaning. Some of the preparations were also claimed to represent 20 and even 30 per cent of fresh posterior lobe material. From our experiments it appears

that the strongest preparation only represents 7 per cent of fresh infundibular material, while most of the others are considerably below our 5 per cent fresh gland extract D. Obviously, the manufacturers either succeeded in getting out only part of the activity of the gland, or lost much of it during the process of manufacture, or else the extracts suffered deterioration during the time intervening between their manufacture and the time of reaching us.

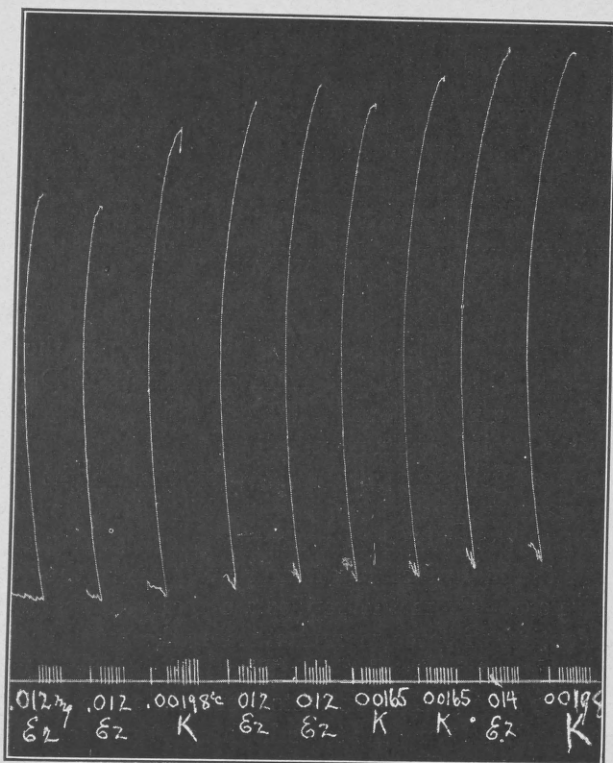
It was not surprising to find that the several commercial extracts vary in activity. Indeed, this was anticipated, since each manufacturer appears to be using his own standard, and there is lack of uniformity. It is difficult to understand, however, the great discrepancy between the activity of the extracts as assayed by us and as is often indicated on the label. To illustrate, two extracts that according to the manufacturers' statement were supposed to represent 20 per cent of fresh gland material, assayed at only a little over 3 and 4 per cent, respectively; and one, claimed to represent the activity of 30 gm. of fresh infundibular substance per 100 c. c. of solvent assayed at less than 1 per cent. It does not seem probable that the time element, within reasonable limits, would be a factor in the deterioration of extracts. Our 5 per cent fresh gland extract D, which was made in July, gave no evidence of deterioration at the time it was examined last, a period of six months.

In the hope of getting some information that might lead to the elimination of what appears to be so much waste, letters of inquiry were sent out to the several manufacturers concerning as much of the details of the manufacture of their products as they could conveniently disclose. From the replies we received we learned that, with the exception of three manufacturers who make their extracts from desiccated material, all the others make their extracts from frozen posterior lobes. The extracts were stated to represent anywhere from nearly 10 per cent to as high as 30 per cent of fresh infundibular substance. The age of the samples at the time of assay varied from 3 to 13 months. Four contained no preservative, while 5 contained some preservative. All were stated to have been sterilized, but the method of sterilization was only indicated in three instances. One of these extracts was stated to have been sterilized by heating in boiling water for 30 minutes, the other under steam pressure at 220° F., and the third by Berkefeld filtration. All were stated to have been physiologically standardized, though neither the method nor the standard were given, with one or two unimportant exceptions.

None of the information given us explained the relatively low activity of the preparations, as found by assay, in comparison with their theoretical strength. Indeed, some of the weakest preparations as

found by assay were stated to represent the highest percentage of infundibular substance.

Since most of the extracts, showing greatest discrepancy in their theoretical potency on the one hand and as found by assay on the other, were stated to have been made from frozen material it seemed possible though not probable that freezing the glands might affect their activity. An experiment was made to determine this point.



TRACING 21.—December 8, 1922. Guinea pig, 240 grams. Assay of 5 per cent extract of fresh frozen glands (K) against infundibular desiccated preparation E2. 0.00198 c. c. K = 0.014 mg. E2, or 1.0 c. c. K = 7.1 mgs. E2. Hence, 1.0 c. c. K =  $7.1 \times 7 = 49.7$  mgs. fresh gland substance.

The whole pituitary glands were placed immediately upon removal in a freezing mixture, and brought into the laboratory in the frozen state. They were then placed in the cold room at a temperature varying from  $-10^{\circ}$  to  $-18^{\circ}$  C. The following day the posterior lobes were dissected out and worked up into a 5 per cent sterilized extract by the method previously described. Tracing 21 shows the result of an assay of this extract (designated K) against infundibular powder E 2. The assay indicated that 1 c. c. of this extract is equal in activity to 7.1 mg. of the powder, or 49.7 mg. of fresh

gland substance, since, as it will be recalled, 1 mg. of the powder is equivalent in activity to 7.0 mg. of fresh infundibular material. Clearly, this experiment indicates that freezing for several hours of the fresh pituitary gland does not affect its oxytocic activity. Freezing over a prolonged period of time might, of course, yield quite different results.

The reason for the poor yield of active principle in the commercial extracts is not altogether clear. However, simple as it is to make a watery or saline extract of the fresh posterior lobe of the pituitary, there are considerable difficulties in both the extraction and the purification, if it is desired to get out the active principles quantitatively. We have already alluded to this point earlier in the paper, and the recent work of Burn and Dale (8) has also emphasized it. While we are not conversant with the problems incidental to the manufacture on a large scale of commercial extracts from frozen material, nevertheless we can readily appreciate that the difficulties there would be far greater than those attending the preparation of an extract on a small scale in a well-equipped laboratory and under the best of technic. On the other hand, the extraction of the active principles from a stable and active infundibular powder, such as we have prepared, can be very easily made quantitatively with the ordinary amount of skill and care possessed by the average laboratory worker. We do not believe the preparation of such a powder on a large scale would be impracticable.

Now the question remains to be answered, what shall be the strength of the pituitary extract that the manufacturers shall be required to make and bring up by prescribed methods of assay to the set standard in order to insure uniformity in potency? Shall they be required to make an extract to correspond in potency to the weakest, such as numbers 2 and 5 or to the strongest as number 3 of the nine specimens we examined? This can not be answered definitely until carefully conducted clinical observations have been made using a definite laboratory unit of measuring potency as a basis. Theoretically, to prescribe one extreme may be as undesirable as to prescribe the other. The preparation which we have found to be the weakest may be ineffective in an emergency such as postpartum hemorrhage when administered in the accustomed dose; while on the other hand the usual dose of the strongest preparation may be altogether excessive for the ordinary therapeutic uses. The strength of a preparation is certainly not an index to its quality. Theoretically, at least, it should be just as easy to make a very strong extract as it is a very weak one, and from our experience we feel certain that this is true practically. Without definite clinical information, it is not possible to state at this time which would be most desirable for average use.

We believe that until such information is made available, which of necessity will have to be based upon cooperative work between the clinician and the laboratory worker, it would seem best at present to recommend that manufacturers make an extract 1.0 c. c. to represent the entire activity of 30 mg. of fresh infundibular substance, or approximately that of 4 mg. of our desiccated infundibular powder. This does not appear to be a very high requirement, should be easy to meet, and, we believe, would be a reasonably sound basis from which to start. Should it prove in time to be too strong or too weak for practical therapeutic uses, the manufacturers could then be asked by general agreement to increase or decrease its potency. This would of necessity eliminate the tendency on the part of some manufacturers to claim superiority for their product on the ground of its high potency, which is misleading and unjustifiable.

#### SUMMARY AND CONCLUSIONS.

The unsatisfactory status of the artificial standards heretofore proposed for the physiological assay of pituitary extracts is discussed.

A method is described for the preparation of an infundibular powder of uniform potency, representing the whole oxytocic and pressor activity of the infundibular lobe of the pituitary gland. The powder has not shown any deterioration within the period of observation of nearly nine months. The active principle or principles are readily extracted quantitatively from the powder by simple manipulation. The oxytocic activity of 1 mg. of this powder is equivalent to that of 7 mg. of fresh infundibular substance.

The results of the physiological assay of nine commercial extracts by the isolated uterus method, using the above powder as a standard, are given. It is shown that there is great variation in the activity of commercial extracts, the strongest having been found eight times as strong as the weakest.

It is proposed that an infundibular powder, such as is described herein, be used as a standard for the assay of commercial extracts by the isolated uterus method, and the recommendation is made that manufacturers be required to make and standardize their extracts to represent the activity of 4 mg. of the standard powder, or its approximate equal, which is 30 mg. of fresh gland substance per c. c. of extract.

It is further suggested that the recommended strength of the commercial extract remain in force until properly conducted clinical observations with carefully standardized extracts make a change desirable.

The following conclusions may also be drawn from the experiments described herein:

1. Infundibular lobes of the pituitary gland from cattle do not show seasonal variations in oxytocic activity.
2. There is no appreciable difference in oxytocic activity of infundibular lobes from steers and from cows.
3. Freezing freshly removed pituitary glands for several hours does not affect their oxytocic activity.
4. The oxytocic activity of properly acidulated extracts is not affected by fractional sterilization in steam at 100° C. Heating in the autoclave at 15 pounds pressure, for even brief periods, causes deterioration in activity.

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## SOME FACTORS CONCERNED IN THE DETERIORATION OF PITUITARY EXTRACTS.<sup>1</sup>

By MAURICE I. SMITH, Pharmacologist, and W. T. McCLOSKEY, Assistant Pharmacologist, Hygienic Laboratory, Washington, D. C.

In a previous communication (3) we reported the results of our assay of several commercial pituitary extracts using as a standard a stable desiccated infundibular powder of uniform potency which was developed in this laboratory.<sup>2</sup> It was pointed out in this report that, aside from the great variation in activity of the commercial extracts examined, their potency, as determined by our assays against the standard powder and calculated in terms of equivalent amount of fresh infundibular substance, was much inferior to what one might expect from the figures indicated on the labels. Thus extracts, purporting to represent 10 to 30 per cent of fresh infundibular material, actually assayed at from 1 to 7 per cent. It would seem that the discrepancy could only be accounted for by one of two factors. First, the extracts may have lost much of their activity during the process of manufacture, or only part of the activity was obtained from the raw material. Second, the extract may have left the manufacturer in full theoretical strength, but deteriorated in activity through time, or other factors attendant upon storage.

Of the two problems at hand the latter is susceptible of solution, since all the factors concerned can be carefully controlled. The former does not readily yield itself to solution, since the method of preparation of the extract is somewhat of a secret with each manufacturer, and the conditons can not be accurately reproduced experimentally.

In this work an attempt has been made to determine the factors that might be concerned in the depreciation in the activity of an infundibular extract through the influence of time, temperature, and light. Some experiments were also made to determine under what conditions raw infundibular material will keep without loss of activity.

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<sup>1</sup> Manuscript submitted for publication October 6, 1923.

<sup>2</sup> Since our last report on the use of the infundibular powder as a standard for the bio-assay of pituitary extracts we have had occasion to prepare three more lots of such material in quantities of 2, 10, and 14 gm. each. These were made during the months of February, March, and August, respectively, and their activity was in nowise different from the seven samples previously reported on.

## THE DETERIORATION OF INFUNDIBULAR EXTRACTS UNDER DIFFERENT CONDITIONS OF STORAGE.

The following experiments were performed: A 5 per cent acidulated aqueous extract of fresh infundibular material was prepared, distributed into ampoules and sterilized by fractional sterilization. This extract was designated D.<sup>3</sup> The greater bulk of this extract was placed in the ice box at a temperature of about 0° C. From time to time assays of this extract were made against the standard powder, by the isolated uterus method of Dale, to determine its oxytocic activity. The details of the method as carried out in this laboratory were described in the paper referred to (3).

Several ampoules of the same extract were placed in the incubator at 37° C., and this extract was likewise assayed at stated intervals, either against the standard powder or against the extract kept in the ice box. Similar assays were carried out at stated intervals upon some ampoules of the same extract kept at a constant temperature of 60° C.

In another series of experiments, quantitative acidulated aqueous extracts were made from the standard infundibular powder and designated M and K 2, respectively. The former was made of the strength of 7.0 mg. of standard powder per cubic centimeter, and the latter was made to contain the activity of 5.0 mg. of standard powder per cubic centimeter. The two extracts were placed in ampoules and sterilized by fractional sterilization at 100° C. Part of extract M was stored on a shelf in the laboratory in diffuse light, the remainder was placed in the incubator at 37° C. Extract K 2 was divided into three lots and stored at 37° C, 45° C., and 60° C., respectively. Assays of these extracts were made from time to time to determine their oxytocic activity, and in many instances experiments were also carried out upon the blood pressure of a dog with a view to ascertaining the effect of storage under different conditions on the pressor activity of the extracts. These experiments were intended not only as a confirmation of the findings by the isolated uterus method, but also as some contributory evidence on the question of identity or nonidentity of the oxytocic and pressor principle or principles in infundibular extracts.

The results of the observations are summarized in Table I. The assays were usually carried out in duplicate, and the figures recorded represent the averages.

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<sup>3</sup> The method of preparation of this extract was described in the previous publication (3), in which experiments are also detailed showing the ratio of activity of this extract and the standard desiccated powder.

TABLE I.—*Effect of storage at different temperatures on the activity of infundibular extracts.*

## ICE BOX TEMPERATURE (ABOUT 0° C.).

Extract No.	Activity in mg. of standard powder.	Age at time of assay.	Activity at time of assay, mg. of standard powder.	Deterioration per cent.
D.....	7.0	1 year.....	7.0	0

## LABORATORY TEMPERATURE. DIFFUSE SUNLIGHT.

M.....	7.0	4 months..... 5 months..... 6 months..... 8 months.....	6.5 6.3 6.7 <sup>2</sup> 6.5	( <sup>3</sup> )
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## INCUBATOR TEMPERATURE (37° C.).

D.....	7.0	1½ months..... 2 months..... 4 months..... 6 months.....	<sup>1</sup> 7.0 <sup>1</sup> 7.0 6.7 <sup>1, 2</sup> 7.0	0 0 0 0
M.....	7.0	3 months.....	<sup>1</sup> 7.0	0
K2.....	5.0	1 month..... 2 months..... 3 months.....	5.0 4.7 <sup>2</sup> 3.9	0 6 22

## INCUBATOR TEMPERATURE (45° C.).

K2.....	5.0	1 week..... 2 weeks..... 1 month..... 2 months..... 3 months.....	<sup>2</sup> 3.9 <sup>2</sup> 4.1 3.8 4.0 3.6	22 18 24 20 28
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## INCUBATOR TEMPERATURE (60° C.).

D.....	7.0	1½ months.....	<sup>2</sup> 1.3	82
K2.....	5.0	3 months.....	<sup>2</sup> 0.2	96

<sup>1</sup> Assayed against same extract kept in the ice box.<sup>2</sup> Assayed also for its pressor activity by the blood-pressure method.<sup>3</sup> Very slight if any deterioration.

Infundibular extract D was prepared in July, 1922, was standardized monthly for one year, and only the last assay, made in July, 1923, is indicated in the table, which shows it to have retained its entire activity. (See tracing 1.)

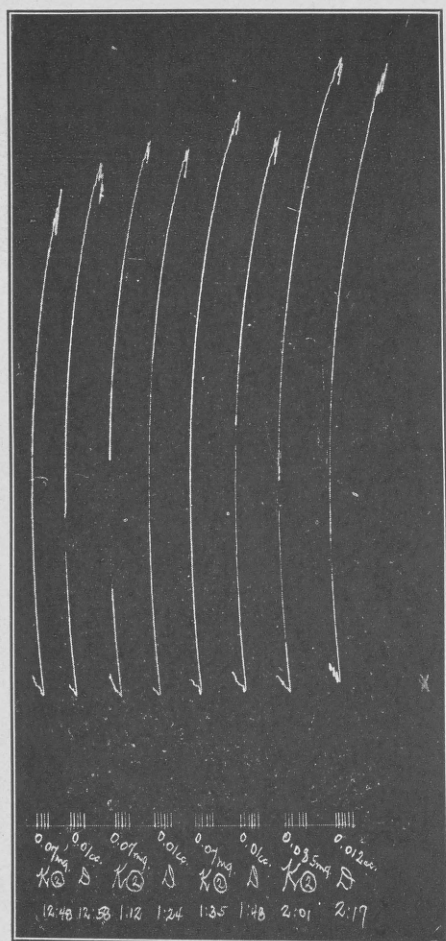
At no time during the year has a notable deviation been observed.

Extract D, kept in the incubator at 37° C. also failed to show any decrease in activity over a period of six months, when tested against the same extract kept in the ice box or against the standard infundibular powder. Similarly, extract M, made from the standard powder, retained its entire activity at 37° C. for 3 months.<sup>4</sup> The same ex-

<sup>4</sup> Tate(4) also has recently found that infundibular extracts of acidity ranging from 0.2 per cent to 2 per cent acetic acid lost none of their oxytocic activity on exposure to a temperature of 38° C. for 25 days.

tract, placed in ampoules and kept on a shelf in the laboratory in diffuse sunlight, retained practically its entire activity over an observation period of eight months.

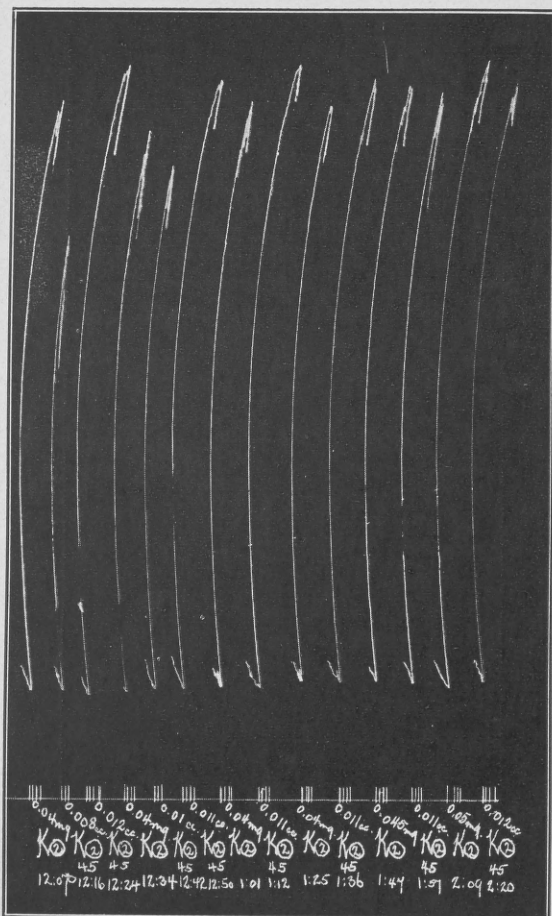
Extract K 2, made in the usual manner from the standard powder, lost very little of its activity at incubator temperature 45° C. The



TRACING 1.—July 12, 1923. Uterus of guinea pig weighing 210 grams. Assay showing no deterioration in oxytocic activity of extract D, which had been kept one year in the ice box (about 0° C.). Result: 0.01 c. c. D = 0.07 mg. K2 (standard powder). Hence, 1 c. c. D = 7.0 mgs. standard powder. (Time in minutes.)

extract remained perfectly clear throughout the period of observation of three months. This finding was so unexpected and interesting that a supplementary assay of the extract for its pressor activity seemed desirable. Tracings 2 and 3 indeed show close conformity of the oxytocic and pressor activity of this extract after remaining

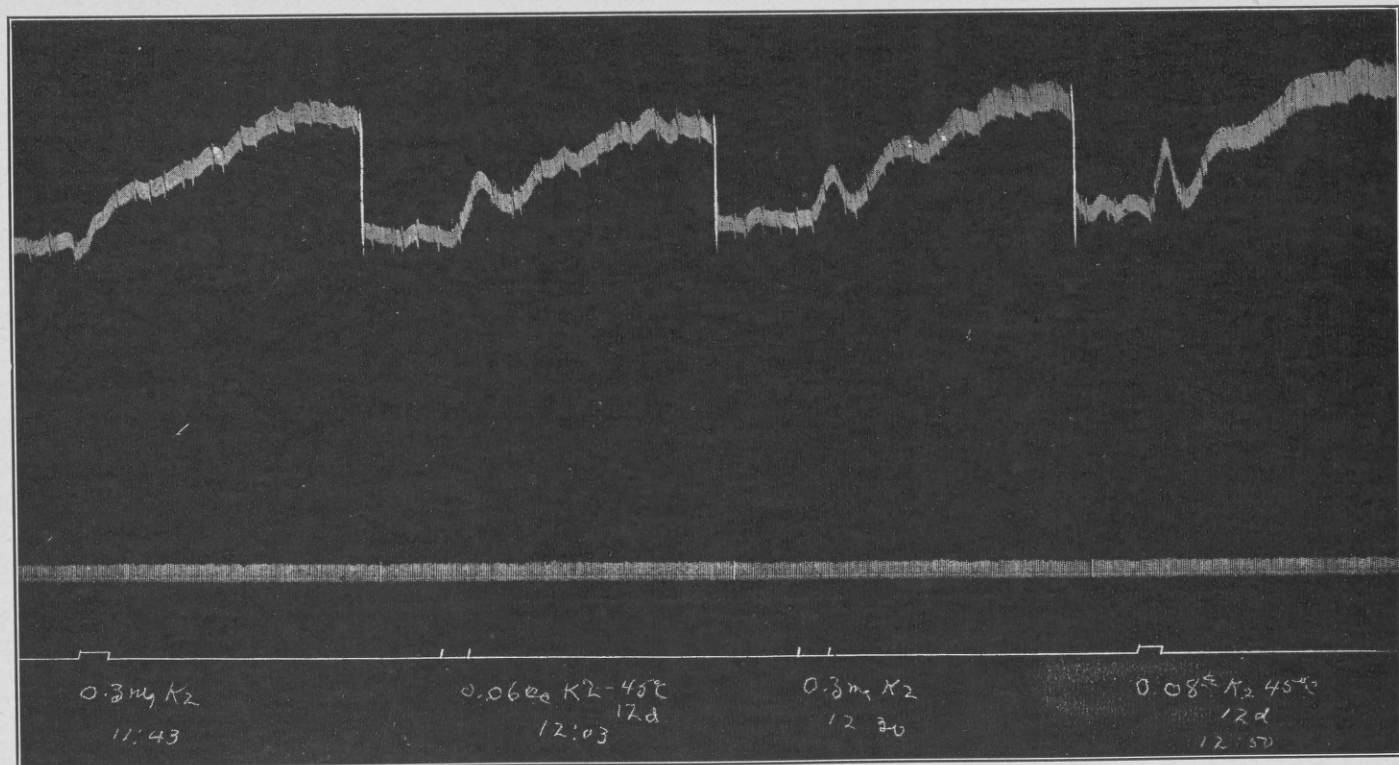
for one to two weeks at a temperature of  $45^{\circ}$  C. The loss in activity at this time was slight, and seemed to remain at about the same level for the entire period of observation of three months. It should be added that this extract, like all the others, was carefully standardized immediately after it was prepared and sterilized, and was



TRACING 2.—June 5, 1923. Uterus of guinea pig weighing 200 grams. Assay of extract K2 kept one week at  $45^{\circ}$  C. (K2-45) against standard infundibular powder K2. Result: 0.011 c. c. K2-45 = 0.045 mg. K2. Hence, 1 c. c. extract K2-45 = 4.1 mgs. standard powder, or 18 per cent deterioration. (Time in minutes.)

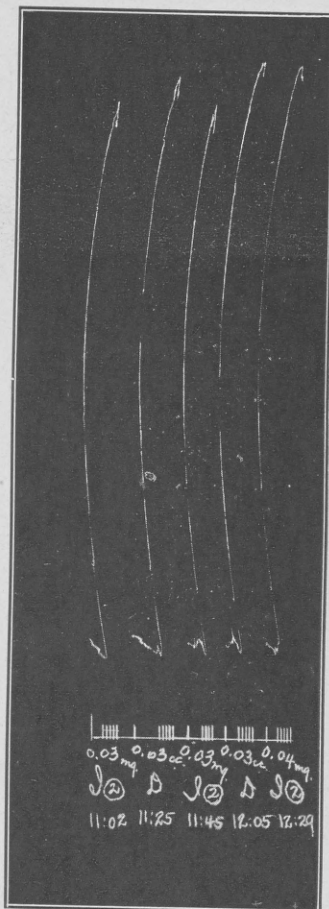
found to have the full theoretical activity, viz, 5 mg. of standard powder per cubic centimeter.

When kept at more elevated temperatures, the extract gradually loses its activity; both pressor and oxytocic activity apparently deteriorating at the same rate, as far as can be judged from the few observations. Extract D, kept in the incubator one and one-half



TRACING 3.—Dog, female; weight 8 kilos.; morphine and chloretone anesthesia; 10 mgs. atropine intravenously; time in seconds. Assay of the pressor activity of extract K-2 kept at 45° C. for 12 days (K2-45) against standard powder K2. Shows that 0.08 c. c. K2-45 is approximately equal to 0.3 mg. standard powder K2, making 1 c. c. of the extract = 3.8 mgs. K2 powder, or about 24 per cent deterioration in pressor activity (cf. Tracing No. 2).

months at 60° C., lost 80 per cent of its oxytocic activity, as shown in tracing 4; and, as nearly as could be determined, the same loss was found in its pressor activity. Extract K 2, kept under the same conditions for three months, retained only a trace of its oxytocic

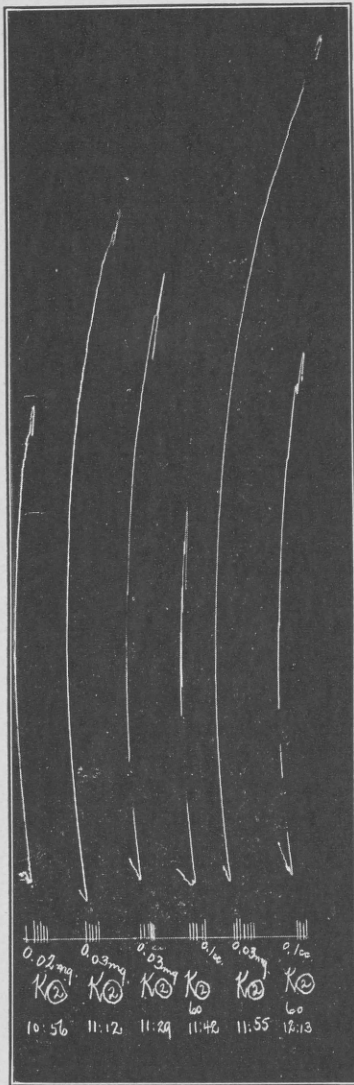


TRACING 4.—March 21, 1923. Uterus of guinea pig weighing 180 grams. Assay showing marked deterioration in oxytocic activity of extract D kept at a temperature of 60° C. for one and one-half months. Result: 0.03 c. c. D = 0.04 mg. standard powder 12. Hence, 1 c. c. D = 1.3 mgs. standard powder 12 or extract D deteriorated to the extent of  $\frac{(7.0 - 1.3) \times 100}{7} = 82$  per cent. (Time in minutes.)

activity, viz, less than 6 per cent; and an indeterminable trace in pressor activity was likewise found by the blood pressure test, as is shown in tracings 5 and 6.

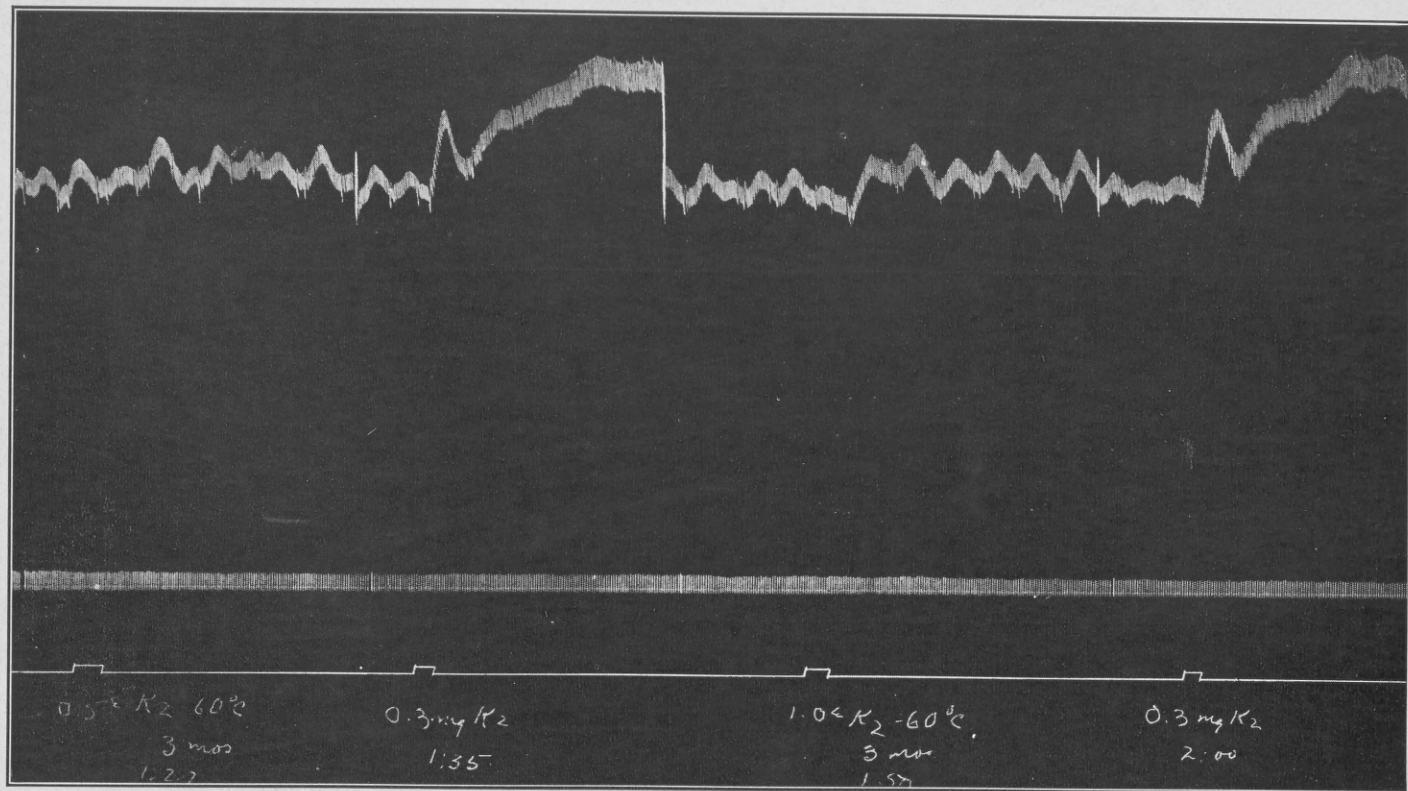
From a practical standpoint, the effect of the higher temperatures on prolonged storage need not concern us, for these conditions probably never prevail. Practically, it need only be emphasized that a

Carefully prepared extract will retain its entire activity for at least a year, if kept in the cold; and further, will suffer no appreciable loss in either oxytocic or pressor activity over fairly long periods



TRACING 5.—June 27, 1923. Uterus of guinea pig weighing 250 grams. Assay showing nearly complete destruction of oxytocic principle in extract K2 kept at 60° C. (K2-60) over a period of three months. Result: 0.1 c. c. K2-60 less than and approximately equal to 0.02 mg. standard powder K2, or 1 c. c. = 0.2 mg. K2, thus showing about 96 per cent deterioration. (Time in minutes.)

of time, if exposed to such temperatures as may prevail during the shipment of the product. Neither does diffuse daylight at room temperature have a deteriorating effect on the extract in ampoules.



TRACING 6.—Dog, female; weight, 8.0 kilos.; morphine and chloretone anesthesia; atropine intravenously. Time in seconds. Shows nearly complete destruction of pressor principle in extract K<sub>2</sub> kept at 60° C. over a period of three months. Note that 1 c. c. extract K<sub>2</sub>-60 shows much less activity than 0.3 mg. standard powder. The extract has thus retained only a trace of its original pressor activity. (Cf. Tracing 5.)

Furthermore, our observations lead us to question the necessity or even the wisdom of using a preservative, as some manufacturers do, provided the extract is made from good material and is carefully sterilized.

The effects of higher temperatures on the activity of infundibular extract are recorded here as information added to our rather meager knowledge of the chemistry of its active principle. The temperature at which marked destruction of the active principle occurs, under the conditions of the experiment, lies within the narrow limits of 45 and 60° C. The material in ampoules kept at 60° C. developed a marked turbidity, while that kept at 45° C. and below remained clear throughout. The gradual and nearly complete destruction of activity at 60° C. reminds us of Abel and Nagayama's observation, that boiling the extract with 0.5 per cent to 1 per cent HCl causes rapid and complete destruction of the pressor and oxytocic activity by hydrolysis (1). It is interesting that a similar process can occur at a much lower temperature and in a medium of 0.25 per cent acetic acid. The process does not appear to be the same, however, for Abel and Nagayama found depressor substances of the histamine type among the end products of hydrolysis; while we could detect no such substances in extracts D and K 2 after exposure for three months to a temperature of 60° C., and at a time when practically all of the oxytocic and pressor activity had disappeared.

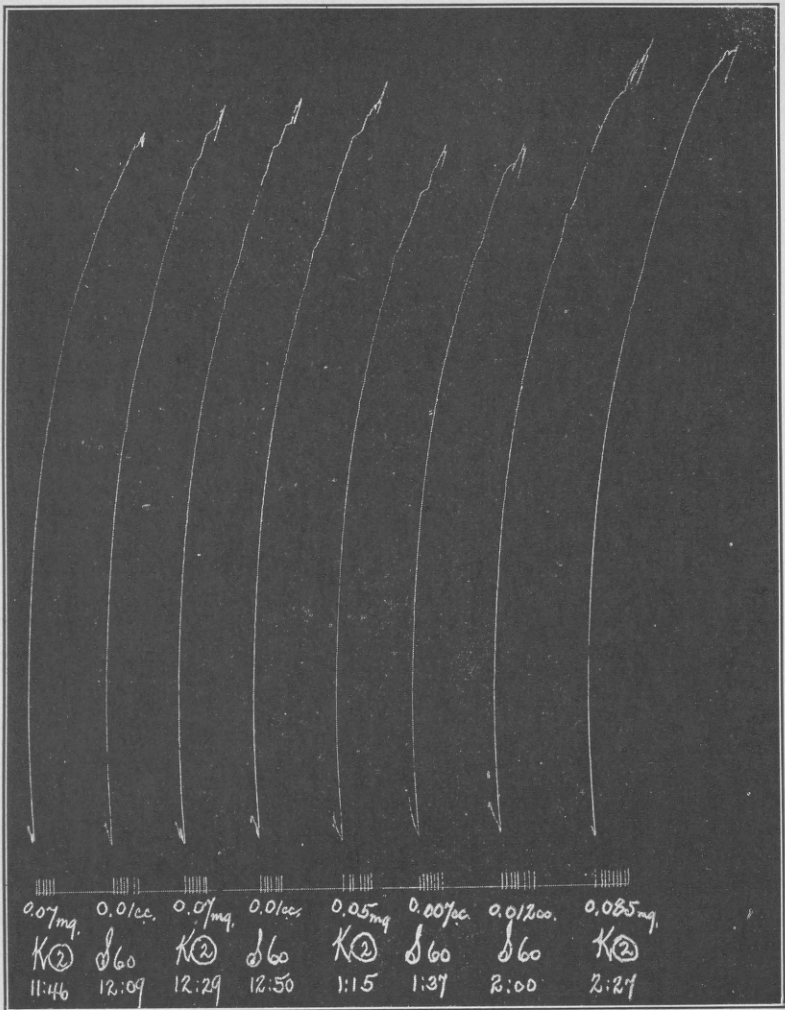
Furthermore, tests carried out for the biuret and Pauly reactions upon extracts D and K 2, at a time when they had practically completely deteriorated in oxytocic and pressor activity, yielded positive reactions and of an intensity indistinguishable from that given by the same extracts kept in the ice box and which had retained their entire activity. Abel and Nagayama, on the other hand, state that their purified extract failed to give the biuret reaction after hydrolysis, though the Pauly reaction remained unaltered.

The observation recorded here, that the oxytocic and pressor activity deteriorate in a quantitatively parallel manner, lends support to the conclusions of Abel and Rouiller (2) concerning the chemical identity of the oxytocic and pressor principle in infundibular extracts.

#### THE EFFECT OF STORAGE OF FRESH INFUNDIBULAR LOBES ON THE ACTIVITY OF EXTRACTS MADE THEREFROM.

The commercial manufacturer of pituitary extracts frequently employs material that has been in cold storage for a variable length of time. Having already shown (3) that freezing of whole pitui-

tary glands for several hours has no effect on the oxytocic activity of the infundibular extract made from such glands, it seemed desir-



TRACING 7.—September 24, 1923. Uterus of guinea pig weighing 240 grams. S60 = 5 per cent extract of frozen fresh infundibular lobes that had been kept for two months at a temperature of  $-10^{\circ}$  C. to  $-17^{\circ}$  C. K2, standard infundibular powder. Result: 0.01 c. c. S60 = 0.07 mg. K2; 0.007 c. c. S60 = 0.05 mg. K2; 0.012 c. c. S60 = 0.085 mg. K2. Hence, 1.0 c. c. S60 = 7.0 mgs. K2. (Time in minutes.)

able to ascertain how long chilled glands may be kept without deteriorating in activity.

The following experiment was performed: The whole pituitary glands of cattle were removed shortly after slaughtering and im-

mediately placed in a freezing mixture. The frozen glands were then brought to the laboratory, the infundibular lobes quickly and carefully dissected out, and the material divided into two lots. One lot was placed in the cold room with a temperature ranging from  $-2^{\circ}$  C. to  $+2^{\circ}$  C., while the other lot was placed in a cold room with a temperature ranging from  $-10^{\circ}$  C. to  $-17^{\circ}$  C. At intervals of two weeks, one month, and two months, 5 per cent acidulated aqueous extracts were carefully made from the respective lots, sterilized, and assayed against the standard infundibular powder. At the end of the two weeks period the material kept at the higher temperature ( $-2^{\circ}$  C. to  $+2^{\circ}$  C.) assayed at nearly full theoretical value (6.3 mg. standard powder activity per cubic centimeter). The material at this time appeared good and presented no evidence of bacterial decomposition. At the end of one month it appeared grayish and showed distinct evidence of some putrefactive change. An extract made of the material at this time assayed at less than one-half the theoretical strength (3.3 mg. standard powder activity per cubic centimeter).

The infundibular lobes kept at the lower temperature ( $-10^{\circ}$  C. to  $-17^{\circ}$  C.) have shown no evidence of deviation from the normal in appearance, and extracts made therefrom at the stated intervals have assayed at full strength. An assay made of the 5 per cent extract of this material at the end of the two-months period showed an oxytocic activity equivalent to 7.0 mg. standard powder per cubic centimeter (see tracing 7).

#### CONCLUSIONS.

1. Infundibular extracts, carefully prepared and sterilized, do not depreciate in activity for at least one year, if kept in the cold room at a temperature of about  $0^{\circ}$  C.

2. Higher temperatures up to  $37^{\circ}$  C. ( $98^{\circ}$  F.), and up to about two months do not affect the oxytocic or pressor activity of sterilized extracts, made either from fresh infundibular lobes or from desiccated standard material. Slight deterioration may occur after this period. The activity of the extract appears to withstand room temperature and diffuse sunlight for eight months without appreciable deterioration.

3. Slight deterioration in activity of the extract occurs at  $45^{\circ}$  C., and nearly complete destruction of its active principle or principles takes place after a period of three month's exposure at  $60^{\circ}$  C. As nearly as can be determined, the deterioration of pressor activity runs parallel to that of the oxytocic activity at the different periods

of exposure at this temperature. This observation lends support to the hypothesis of the chemical identity of the oxytocic and pressor principle.

4. Frozen fresh infundibular lobes retain their oxytocic activity for at least two months, if kept at a temperature of  $-10^{\circ}$  to  $-17^{\circ}$  C. At a temperature of about  $0^{\circ}$  C., the activity is not retained longer than about two weeks.

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- (3) Smith, M. L., and McClosky, W. T.: Hyg. Lab. Bull. 138, p. 281.
- (4) Tate, G.: Pharm. J., London, 1921, v. 106, p. 486.

## HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

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The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

Of the bulletins published by the laboratory since its establishment copies of the following are available for distribution and may be obtained without cost by applying to the Surgeon General, United States Public Health Service, Washington D. C.

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No. 65.—Facts and problems of rabies. By A. M. Stimson.

No. 73.—The effect of a number of derivatives of choline and analogous compounds on the blood pressure. By Reid Hunt and R. de M. Taveau.

No. 78.—Report No. 4 on the origin and prevalence of typhoid fever in the District of Columbia (1909). By L. L. Lumsden and John F. Anderson. (Including articles contributed by Thomas B. McClintic and Wade H. Frost.)

No. 81.—Tissue proliferation in plasma medium. By John Sundwall.

No. 89.—Sewage pollution of interstate and international waters with special reference to the spread of typhoid fever. VI. The Missouri River from Sioux City to its mouth. By Allan J. McLaughlin.

No. 95.—Laboratory studies on tetanus. By Edward Francis.

No. 97.—Some further siphonaptera. 2. A further report on the identification of some siphonaptera from the Philippine Islands. 3. The taxonomic value of the copulatory organs of the females in the order of siphonaptera. By Carroll Fox.

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No. 101 (Reprint a).—IV. The sterilization of dental instruments. By H. E. Hasseltine.

No. 102.—I. Digitalis standardization. The physiological valuation of fat-free digitalis and commercial digitalin. By George B. Roth. II. Preliminary observations on metabolism in pellagra. By Andrew Hunter, Maurice H. Givens, and Robert C. Lewis.

No. 103.—I. Chemical changes in the central nervous system as a result of restricted vegetable diet. By Mathilde L. Koch and Carl Voegtlin. II. Chemical changes in the central nervous system in pellagra. By Mathilde L. Koch and Carl Voegtlin.

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producing diet. By M. X. Sullivan and K. K. Jones. III. Biological study of a diet resembling the Rankin farm diet. By M. X. Sullivan. IV. Feeding experiments with the Rankin farm pellagra-producing diet. By M. X. Sullivan.

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